

09/24/08

AJ/16/08
JGD

PTO/SB/21 (04-07)

Approved for use through 09/30/2007. OMB 0851-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

OTPE
TRANSMITTAL
FORM
SEP 20, 2007
PATENT & TRADEMARK OFFICE
for all correspondence after initial filing)

		Application Number	09/445,328
		Filing Date	December 7, 1999
		First Named Inventor	Kuber T. Sampath et al.
		Art Unit	1647 (Confirmation No. 9813)
		Examiner Name	David S. Romeo
Total Number of Pages in This Submission		Attorney Docket Number	JJJ-P01-514

ENCLOSURES (Check all that apply)

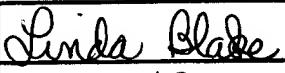
<input type="checkbox"/> Fee Transmittal Form	<input type="checkbox"/> Drawing(s)	<input type="checkbox"/> After Allowance Communication to TC
<input type="checkbox"/> Fee Attached	<input type="checkbox"/> Licensing-related Papers	<input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences
<input type="checkbox"/> Amendment/Reply	<input type="checkbox"/> Petition	<input checked="" type="checkbox"/> Appeal Communication to TC (Amended Reply Brief)
<input type="checkbox"/> After Final	<input type="checkbox"/> Petition to Convert to a Provisional Application	<input type="checkbox"/> Proprietary Information
<input type="checkbox"/> Affidavits/declaration(s)	<input type="checkbox"/> Power of Attorney, Revocation	<input type="checkbox"/> Status Letter
<input type="checkbox"/> Extension of Time Request	<input type="checkbox"/> Change of Correspondence Address	<input type="checkbox"/> Other Enclosure(s) (please Identify below):
<input type="checkbox"/> Express Abandonment Request	<input type="checkbox"/> Terminal Disclaimer	Copy of Exhibit D, F, I, K, L, and M
<input type="checkbox"/> Information Disclosure Statement	<input type="checkbox"/> Request for Refund	<input type="checkbox"/> Return Receipt Postcard
<input type="checkbox"/> Certified Copy of Priority Document(s)	<input type="checkbox"/> CD, Number of CD(s) _____	
<input type="checkbox"/> Reply to Missing Parts/ Incomplete Application	<input type="checkbox"/> Landscape Table on CD	
<input type="checkbox"/> Reply to Missing Parts under 37 CFR 1.52 or 1.53		
<p>Remarks The Director is hereby authorized to charge payment of any fees required in connection with filing of these papers to Deposit Account No. 18-1945, Order No. JJJ-P01-514. A duplicate copy of this letter is transmitted herewith.</p>		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	Ropes & Gray LLP		
Signature			
Printed name	Erika Takeuchi		
Date	September 20, 2007	Reg. No.	55,661

CERTIFICATE OF EXPRESS MAIL (EXPRESS MAIL LABEL NO. EM 016026310 US)

I hereby certify that this correspondence is being deposited with the United States Postal Service "EXPRESS MAIL POST OFFICE TO ADDRESSEE" service under 37 C.F.R. §1.10 on the date indicated above and is addressed to Mail Stop: Appeal Brief Patents, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450:

Signature			
Typed or printed name	Linda Blake	Date	September 20, 2007

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

American LegalNet, Inc.
www.FormsWorkflow.com

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as Express Mail, Airbill No. EM 016026310 US, in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Dated: September 20, 2007 Signature: Linda Blake
(Linda Blake)

Docket No.: JJJ-P01-514
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

SEP 20 2007
O I P E
I A P E S
P A T E N T S
T R A D E M A R K S
S E R V I C E S
In the Patent Application of:
Sampath et al.

Application No.: 09/445,328

Confirmation No.: 9813

Filed: December 7, 1999

Art Unit: 1647

For: THERAPIES FOR ACUTE RENAL FAILURE

Examiner: D. S. Romeo

APPEAL BRIEF

MS Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

This responds to the Notice of Non-Compliant Appeal Brief dated August 21, 2007. The deadline for filing an amended Appeal Brief is one month from the date of mailing of the Notice, or September 20, 2007. Accordingly, this amended Appeal Brief is timely filed, and is in furtherance of the Notice of Appeal filed on December 8, 2006.

Please also note that the real party of interest has been amended because of the termination of a license agreement. There is no longer a licensee as a party of interest.

Payment of fees required under § 41.20(b)(2) is authorized in the accompanying TRANSMITTAL OF APPEAL BRIEF.

This brief contains items under the following headings as required by 37 C.F.R. § 41.37:

- I. Real Party In Interest
- II. Related Appeals and Interferences
- III. Status of Claims

- IV. Status of Amendments
- V. Summary of Claimed Subject Matter
- VI. Grounds of Rejection to be Reviewed on Appeal
- VII. Argument
- VIII. Claims Appendix
- IX. Evidence Appendix
- X. Related Proceedings Appendix

I. REAL PARTY IN INTEREST

The real party in interest for this appeal is as follows:

Curis, Inc., the owner of the application.

II. RELATED APPEALS, INTERFERENCES, AND JUDICIAL PROCEEDINGS

To the best of the knowledge of the undersigned, there are no other appeals, interferences or judicial proceedings known to the Appellant, the Appellant's legal representative, or the above-noted real party of interest that will directly affect or be directly affected by, or have a bearing on, the Board's decision in this appeal.

III. STATUS OF CLAIMS

A. Total Number of Claims in Application

There are 45 claims pending in application.

B. Current Status of Claims

1. Claims canceled: 1, 3, 4, 7, 10, 13, 39-52
2. Claims withdrawn from consideration but not canceled: 21, 22, 25, and 28-34
3. Claims pending: 2, 5, 6, 8, 9, 11, 12, 14-20, 23, 24, 26, 27, 35-38, and 53-65
4. Claims allowed: None
5. Claims objected: None
6. Claims rejected: 2, 5, 6, 8, 9, 11, 12, 14-38, and 53-65

C. Claims On Appeal

The claims on appeal are claims 2, 5, 6, 8, 9, 11, 12, 14-38, and 53-65.

IV. STATUS OF AMENDMENTS

An amendment was filed on December 8, 2006 in response to the Final Office Action

dated September 21, 2006. The Examiner entered the amendment as indicated in the Advisory Action dated January 18, 2007.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Applicants provide the following concise summary of the subject matter defined in each of the independent claims involved in the appeal, with appropriate page and line numbers referring to the cited portions of the originally-filed specification:

Claim 2

The methods and compositions of this invention capitalize in part upon the discovery that certain proteins of eukaryotic origin, defined herein as OP/BMP renal therapeutic agents, and including members of the osteogenic protein/bone morphogenetic protein (OP/BMP) family of proteins, may be used in the treatment of subjects in, or at risk of, acute renal failure. (page 2, line 33-36). Useful renal therapeutic agents include polypeptides, or functional variants of polypeptides, comprising at least the C-terminal six-or seven-cysteine domain of a mammalian protein selected from the group consisting of OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, BMP9, and proteins which exhibit at least 70% or, more preferably, 75% or 80% amino acid sequence homology with the amino acid sequence of the seven-cysteine domain of human OP-1; and are (a) capable of inducing chondrogenesis in the Reddi-Sampath ectopic bone assay (Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA) 78:7599-7603) or a substantially equivalent assay, (b) capable of significantly preventing, inhibiting, delaying or alleviating the permanent or progressive loss of renal function which may result from acute renal failure in a standard animal model of acute renal failure, or (c) capable of causing a clinically significant improvement in a standard marker of renal function when administered to a mammal in, or at risk of, acute renal failure. (page 2, line 36 to page 3, line 10). The renal therapeutic agents of the present invention may be evaluated for their therapeutic efficiency in causing a clinically

significant improvement in a standard marker of renal function when administered to a mammalian subject. (page 11, lines 5-7).

Claim 53

The methods and compositions of this invention capitalize in part upon the discovery that certain proteins of eukaryotic origin, defined herein as OP/BMP renal therapeutic agents, and including members of the osteogenic protein/bone morphogenetic protein (OP/BMP) family of proteins, may be used in the treatment of subjects in, or at risk of, acute renal failure. (page 2, line 33-36). The renal therapeutic agents useful herein include therapeutically effective proteins in which the amino acid sequences comprise a sequence sharing at least 60% amino acid sequence identity, and preferably, 65% or 70% identity with the C-terminal seven cysteine domain present in the active forms of human OP-1. (page 9, lines 9-13). The renal therapeutic agents of the present invention may be evaluated for their therapeutic efficiency in causing a clinically significant improvement in a standard marker of renal function when administered to a mammalian subject. (page 11, lines 5-7)

Claim 58

The methods and compositions of this invention capitalize in part upon the discovery that certain proteins of eukaryotic origin, defined herein as OP/BMP renal therapeutic agents, and including members of the osteogenic protein/bone morphogenetic protein (OP/BMP) family of proteins, may be used in the treatment of subjects in, or at risk of, acute renal failure. (page 2, line 33-36). The renal therapeutic agents useful herein include therapeutically effective proteins in which the amino acid sequences comprise a sequence sharing at least 60% amino acid sequence identity, and preferably, 65% or 70% identity with the C-terminal seven cysteine domain present in the active forms of human OP-1. (page 9, lines 9-13). Useful renal therapeutic agents include polypeptides, or functional variants of

polypeptides, comprising at least the C-terminal six-or seven-cysteine domain of a mammalian protein selected from the group consisting of OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, BMP9, and proteins which exhibit at least 70% or, more preferably, 75% or 80% amino acid sequence homology with the amino acid sequence of the seven-cysteine domain of human OP-1; and are (a) capable of inducing chondrogenesis in the Reddi-Sampath ectopic bone assay (Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA) 78:7599-7603) or a substantially equivalent assay, (b) capable of significantly preventing, inhibiting, delaying or alleviating the permanent or progressive loss of renal function which may result from acute renal failure in a standard animal model of acute renal failure, or (c) capable of causing a clinically significant improvement in a standard marker of renal function when administered to a mammal in, or at risk of, acute renal failure. (page 2, line 36 to page 3, line 10). The renal therapeutic agents of the present invention may be evaluated for their therapeutic efficiency in causing a clinically significant improvement in a standard marker of renal function when administered to a mammalian subject. (page 11, lines 5-7). Generally speaking, acute renal failure may be due to pre-renal, post-renal, or intrinsic renal causes. (Page 1, lines 20-21). As used herein, pre-renal causes of acute renal failure include decreased cardiac output, hypovolemia, volume redistribution, and altered vascular resistance. (page 4, lines 29-31).

Claim 61

The methods and compositions of this invention capitalize in part upon the discovery that certain proteins of eukaryotic origin, defined herein as OP/BMP renal therapeutic agents, and including members of the osteogenic protein/bone morphogenetic protein (OP/BMP) family of proteins, may be used in the treatment of subjects in, or at risk of, acute renal failure. (page 2, line 33-36). The renal therapeutic agents useful herein include therapeutically effective proteins in which the amino acid sequences comprise a sequence sharing at least 60% amino acid

sequence identity, and preferably, 65% or 70% identity with the C-terminal seven cysteine domain present in the active forms of human OP-1. (page 9, lines 9-13). Useful renal therapeutic agents include polypeptides, or functional variants of polypeptides, comprising at least the C-terminal six-or seven-cysteine domain of a mammalian protein selected from the group consisting of OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, BMP9, and proteins which exhibit at least 70% or, more preferably, 75% or 80% amino acid sequence homology with the amino acid sequence of the seven-cysteine domain of human OP-1; and are (a) capable of inducing chondrogenesis in the Reddi-Sampath ectopic bone assay (Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA) 78:7599-7603) or a substantially equivalent assay, (b) capable of significantly preventing, inhibiting, delaying or alleviating the permanent or progressive loss of renal function which may result from acute renal failure in a standard animal model of acute renal failure, or (c) capable of causing a clinically significant improvement in a standard marker of renal function when administered to a mammal in, or at risk of, acute renal failure. (page 2, line 36 to page 3, line 10). The renal therapeutic agents of the present invention may be evaluated for their therapeutic efficiency in causing a clinically significant improvement in a standard marker of renal function when administered to a mammalian subject. (page 11, lines 5-7). Generally speaking, acute renal failure may be due to pre-renal, post-renal, or intrinsic renal causes. (Page 1, lines 20-21). As used herein, pre-renal causes of acute renal failure include decreased cardiac output, hypovolemia, volume redistribution, and altered vascular resistance. (page 4, lines 29-31). Administration is expected to be continuous or frequent (e.g., daily) during the period of acute renal failure, typically 1-3 weeks, but may also be continued for several weeks or months after the acute phase. (page 3, lines 15-17).

Claim 64

The methods and compositions of this invention capitalize in part upon the

discovery that certain proteins of eukaryotic origin, defined herein as OP/BMP renal therapeutic agents, and including members of the osteogenic protein/bone morphogenetic protein (OP/BMP) family of proteins, may be used in the treatment of subjects in, or at risk of, acute renal failure. (page 2, line 33-36). The renal therapeutic agents useful herein include therapeutically effective proteins in which the amino acid sequences comprise a sequence sharing at least 60% amino acid sequence identity, and preferably, 65% or 70% identity with the C-terminal seven cysteine domain present in the active forms of human OP-1. (page 9, lines 9-13). Useful renal therapeutic agents include polypeptides, or functional variants of polypeptides, comprising at least the C-terminal six-or seven-cysteine domain of a mammalian protein selected from the group consisting of OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, BMP9, and proteins which exhibit at least 70% or, more preferably, 75% or 80% amino acid sequence homology with the amino acid sequence of the seven-cysteine domain of human OP-1; and are (a) capable of inducing chondrogenesis in the Reddi-Sampath ectopic bone assay (Sampath and Reddi (1981), Proc. Natl. Acad. Sci. (USA) 78:7599-7603) or a substantially equivalent assay, (b) capable of significantly preventing, inhibiting, delaying or alleviating the permanent or progressive loss of renal function which may result from acute renal failure in a standard animal model of acute renal failure, or (c) capable of causing a clinically significant improvement in a standard marker of renal function when administered to a mammal in, or at risk of, acute renal failure. (page 2, line 36 to page 3, line 10). The renal therapeutic agents of the present invention may be evaluated for their therapeutic efficiency in causing a clinically significant improvement in a standard marker of renal function when administered to a mammalian subject. (page 11, lines 5-7). Generally speaking, acute renal failure may be due to pre-renal, post-renal, or intrinsic renal causes. (Page 1, lines 20-21). As used herein, pre-renal causes of acute renal failure include decreased cardiac output, hypovolemia, volume redistribution, and altered vascular resistance. (page 4, lines 29-31). In some cases, however, the subjects

may present with other symptoms (e.g. osteodystrophy) for which renal therapeutic agent treatment would be indicated. (page 12, lines 5-6).

Although those teachings are summarized above, the Board is strongly urged to study the specification before considering the rejections on appeal.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The two grounds of rejection to be reviewed on appeal are: whether two groups of claims, first being independent claims 2 and 53 and second being independent claims 58, 61, and 64, each satisfies the non-obviousness requirement of 35 U.S.C. 103(a) over cited references.

Claims 2 and 53 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Kelly (J. Clin. Invest. 1996 Feb 15;97(4):1056-63) in view of Kuberasampath (WO 93/04692) and Lefer (J Mol Cell Cardiol. 1992 Jun; 24(6):585-93).

Claims 2, 58, 61 and 64 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Kelly (J. Clin. Invest. 1996 Feb 15;97(4):1056-63) in view of Kuberasampath (WO 93/04692), Lefer (J. Mol. Cell. Cardiol. 1992 Jun;24(6):585-93), further in view of Anderson (Chapter 275, in Harrison's Principles of Internal Medicine, 1980) and Brady (Chapter 236, in Harrison's Principles of Internal Medicine, 1994).

VII. ARGUMENT

For the convenience of the Board, Table A below is provided indicating the relationship between the elements of the five independent claims (claims 2, 53, 58, 61, and 64) under appeal.

Table A: Comparison of Claim Elements of Claims under Appeal

Claims	Agent	Cause of Acute Renal Failure	Agent Administration	Subjects being Treated
2	70% homologous to OP-1 Seven-Cys Domain	ANY	ANY	ANY
53	60% identical to OP-1 Seven-Cys Domain	ANY	ANY	ANY
58	70% homologous <u>OR</u> 60% identical to OP-1 Seven-Cys Domain	Pre-Renal	ANY	ANY
61	70% homologous <u>OR</u> 60% identical to OP-1 Seven-Cys Domain	Pre-Renal	Continuously for 1-3 weeks	ANY
64	70% homologous <u>OR</u> 60% identical to OP-1 Seven-Cys Domain	Pre-Renal	ANY	Afflicted with Osteodystrophy

Claims 2 and 53 relate to any form of acute renal failure, and claim 58 recites pre-renal causes of acute renal failure. However, the examination of claims 2 and 53 were carried out based on acute renal failure as including arising from pre-renal cause, which is an explicit claim limitation in claim 58. Further, the ground for rejection of claims 2 and 53 is alleged obviousness over Kelly in view of Kubersampath and Lefer. The grounds for rejection of claim 2 and 58 is alleged obviousness over Kelly in view of Kubersampath and Lefer, further in view of Anderson and Brady. Appellants intend to show that none of these claims are obvious against cited references, by showing claim 58 is not obvious even when all cited references are combined. As a result, Appellants have grouped claims 2, 53 and 58 for the appeal as standing and falling together.

Claims 61 and 64 will be argued separately since they recite at least one element not found in any one of claims 2, 53, or 58. Nevertheless, if the Board agrees with Appellants that claim 58 is nonobvious over the cited references, then claims 61 and 64 should also be deemed nonobvious since they incorporate all the elements found in claim 58.

CLAIM 2, 53, and 58

The Examiner rejects claim 2, 53, and 58 as being allegedly obvious over Kelly (J. Clin. Invest. 1996 Feb 15;97(4):1056-63) ("Kelly") in view of Kuberasampath (WO 93/04692) ("Kuberasampath") and Lefer (J. Mol. Cell. Cardiol. 1992 Jun; 24(6):585-93) ("Lefer"). Claim 58 is rejected with regard to pre-renal cause in further view of Anderson (Chapter 275, in Harrison's Principles of Internal Medicine, 1980) ("Anderson") and Brady (Chapter 236, in Harrison's Principles of Internal Medicine, 1994) ("Brady").

The Examiner's argument for rejecting claim 2, 53, and 58 may be outlined as follows:

- (1) Kelly suggests that agents that block ICAM-adhesiveness or that block polymorphonuclear cell (PMC) activity might be effective in treating acute renal failure.
- (2) Kuberaampath teaches that the morphogen OP-1 is an anti-inflammatory agent that blocks ICAM adhesiveness.
- (3) Lefer teaches that OP-1 is an anti-inflammatory agent that inhibits PMC activity.
- (4) Therefore, one skilled in the art would expect that the anti-inflammatory OP-1 would be successful in treating acute renal failure.

The Examiner, then, assumes that if agent X is known to reduce inflammation, then one skilled in the art would reasonably expect that agent X would be effective in treating acute renal failure. Based on this central assumption, the Office Action concludes that since OP-1 is allegedly effective in treating inflammation then one skilled in the art would reasonably expect OP-1 to be effective in treating acute renal failure.

As will be shown below, this argument fails because at the time the subject application was filed, anti-inflammatory agents were known to *decrease* renal function, or even to cause outright renal failure, when administered to subjects. In particular, Transforming Growth Factor Beta 1(TGF β 1), Cyclosporin A (CsA) and Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) were documented in the scientific and medical literature to *decrease* renal function when administered to a subject. This clearly teaches away from use of anti-inflammatories in the treatment of acute renal failure. Accordingly, one skilled on the art would have expected that administration of the anti-inflammatory OP-1 to a mammal afflicted with acute renal failure would have aggravated, not improved, renal function in the mammal. In other words, one skilled

in the art would not have had a reasonable expectation that OP-1, or other morphogens, would be effective in treating acute renal failure.

(1) A Reasonable Expectation of Success is Lacking for the Use of OP-1 to Improve Renal Function in Subjects Afflicted with Acute Renal Failure

MPEP 706.02(j) sets forth three basic criteria needed to establish a *prima facie* case of obviousness: 1) the prior art references must teach or suggest all the claim limitations; 2) some motivation or suggestion, either found in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to combine or modify the references must be present; and 3) a reasonable expectation of success.

At least the third prong is not satisfied in this case. The Examiner has failed to show why one skilled in the art would have ignored the scientific literature documenting the adverse renal effects of anti-inflammatory agents, and why he would have selected the anti-inflammatory agent OP-1 to improve renal function in a subject with renal dysfunction (i.e. with acute renal failure). In fact, one skilled in the art would have expected that the morphogen OP-1 would not only fail to improve renal function in a subject afflicted with acute renal failure, but also that it would aggravate the renal dysfunction. The skilled artisan would not have expected OP-1 to be the exception among anti-inflammatory agents.

(2) Anti-Inflammatory Drugs were Known to be Detrimental to Renal Function

At the time the application was filed, it was well-documented in the scientific literature that anti-inflammatory agents reduced, rather than improved, renal function.

On pages 10-16 of the Amendment filed on November 12, 2004, Applicants established that Transforming Growth Factor Beta 1(TGF β 1), Cyclosporin A (CsA) and Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) were known at the time the subject application was filed to be both (i) anti-inflammatory agents which inhibit ICAM adhesiveness, and (ii) *detrimental* to renal function. The November 12, 2004 amendment included thirteen scientific publications, as Exhibits A-M, documenting the anti-inflammatory and the renal-adverse side effects of these three agents. Rather than reproducing this section of the previous office action in this appeal

brief, Appellants provide a summary of the documented adverse renal effects of these three agents in Table B below. The Board is nevertheless encouraged to review the arguments and the Exhibits as provided in the November 12, 2004 amendment in their entirety.

Table B. Documented adverse kidney effects of anti-inflammatory agents

Agent	Exhibit #	Finding
TGF β 1	D	"Recent studies show that TGF-beta overexpression in experimental and human kidney diseases leads to progressive glomerular and tubulointerstitial scarring and renal failure," (Abstract) "New therapies may prevent progressive fibrosis in chronic kidney disease by suppressing the action of TGF-beta." (Abstract)
	F	"Overproduction of TGF-beta is the cause of pathologic matrix accumulation in the nephritic glomeruli" (Abstract) "Studies of humans with glomerulonephritis and diabetic nephropathy also strongly implicated TGF-beta in the pathogenesis of glomerular matrix build-up" (Abstract)
CsA	I	"Cyclosporine A causes an acute reduction in GFR." (Abstract) (GFR stands for Glomerular Filtration Rate, a primary measure of renal function
NSAIDs	K	"Approximately 1-5% of people who are exposed to a nonsteroidal anti-inflammatory drug (NSAID) will manifest one of a variety of renal function abnormalities ... Renal abnormalities include fluid and electrolyte disturbances, acute deterioration of renal function, nephritic syndrome with interstitial nephritis, and papillary necrosis" (Page 588, columns 1-2) "from the clinical point of view, the most worrisome renal side effect of NSAIDs is hemodynamically mediated acute renal failure, which occurs in individuals with pre-existing reduced renal blood perfusion" (Page 588, column 2)
	L	"Patients with pre-existing risk factors are susceptible to potentially life-threatening toxicities [from NSAIDs], including acute renal failure (ARF) and serious fluid and electrolyte disorders" (page S-61, column 1)
	M	"Among persons with normal renal function, who have no other risk factors (dehydration) for an acute hemodynamic effect, there is no risk. However, NSAID administration to susceptible persons may cause decrements in renal plasma flow and glomerular filtration rate within hours" (Abstract)

(3) One skilled in the Art Would Have Expected that Administration of OP-1 to a Mammal

Afflicted with Acute Renal Failure Would Reduce, not Increase, Renal Function

OP-1 shares two key properties with TGF β 1, CsA and NSAIDs: (i) it decreases ICAM

adhesiveness and (ii) it decreases PMC activity. OP-1 and TGF β 1 are also members of the TGF β superfamily of growth factors. The Examiner has focused exclusively on OP-1's anti-inflammatory property as the key attribute in making it a seemingly successful candidate for treating Acute Renal Failure (ARF).

But at the time the application was filed, anti-inflammatory agents were documented to actually cause renal dysfunction, especially in subjects with already impaired renal function. One skilled in the art would have expected that OP-1, just like its counterpart anti-inflammatory agents TGF β 1, CsA and NSAIDs, would further impair renal function in a subject afflicted with acute renal failure. One skilled in the art would have expected that administration of the anti-inflammatory OP-1 polypeptide, based on its anti-inflammatory and neutrophil adhesion-inhibiting properties that it shares with NSAIDs, would reduce, rather than increase, renal function. If anything, the documented anti-renal effects of anti-inflammatory agents taught away from administering anti-inflammatory agents, such as OP-1, to subjects with impaired renal function.

While having the burden of proof, the Examiner has failed to establish why one skilled in the art would have made OP-1 the exception amongst anti-inflammatory agents. He has failed to show why one would have expected OP-1 to be the anomaly and to actually improve renal function where other anti-inflammatory agents failed. The burden of going forward was and is on the Examiner to overcome the presumption of lack of reasonable expectation of success legitimately established by applicant using documentary evidence during prosecution. Because he has failed to do so, he has failed to establish a *prima facie* case of obviousness in accordance with MPEP 706.02(j).

(4) The Examiner's Counterarguments Fail to Address Why OP-1 Would Have Been Expected to Be the Exception Among Anti-inflammatory Agents

In response to Appellants arguments, the Examiner alleges that there is no evidence of record that OP-1 possesses any of the renal side effects of TGF β 1, CsA or NSAIDS. The Examiner claims that applicants have not met a burden of proof in providing a nexus between (i) anti-inflammatory agents inhibiting ICAM adhesiveness and (ii) anti-inflammatory agents being

detrimental to acute renal function. But the burden is on the Examiner, not on applicants, to establish the third prong of the *prima facie* case of obviousness. It is the Examiner who must present evidence why one skilled on the art would have expected a fourth anti-inflammatory agent (OP-1) to be the exception among anti-inflammatories – to show why a fourth anti-inflammatory would be effective in treating acute renal failure when the three others anti-inflammatory agents impair renal function. The Examiner wants Appellants to provide evidence that OP-1 had the adverse renal effects of the other anti-inflammatory agents. But this is impossible because Appellants discovered that, contrary to expectation, OP-1 could improve renal function.

With regard to Claim 2 and 58, adding Anderson and Brady as references does not cure the defect of the Examiner's argument based on Kelly in view of Kuberasampath and Lefer. Appellants have shown that Kelly in view of Kuberasampath and Lefer, regardless of whether in further view of Anderson and Brady, does not make the claims at issue obvious.

The Examiner has made some additional rebuttals on previous Office Actions, but none of them address the heart of the matter: why would OP-1 be the exception among anti-inflammatory agents? Some of these rebutting arguments are as follows:

(i) The Examiner alleges that despite the evidence showing the ineffectiveness of anti-inflammatories in treating ARF, one could not have known for sure whether OP-1 would fail in treating ARF until it was actually tested. The Examiner's position turns the test for obviousness on its head. The standard is the reasonable expectation that the invention would work successfully, and not whether there was the infinitesimal chance that OP-1 might improve renal function contrary to expectation. Indeed, since there is no evidence supplied by the Examiner that OP-1 would be effective in treating ARF, and documentary evidence shows that other anti-inflammatories were ineffective, there is no *prima facie* case of obviousness.

(ii) The Examiner points to differences between OP-1 and TGF β 1 in bone formation to suggest that the two molecules might have different biological properties in treating other organs. The question, however, is not whether the possibility exists, no matter how small, that two compounds can have different properties. The question is what properties one skilled in the art would have expected the morphogens to have and why one would have expected OP-1 to be an

exception. Merely pointing out that OP-1 is a different compound than TGF β 1, CsA or an NSAID, proves nothing. TGF β 1, CsA or an NSAID all have different structures from each other yet they all reduce inflammation and reduce renal function. The common teaching of such prior art is that anti-inflammatories generally have an adverse effect on renal function. The claimed invention runs counter to conventional wisdom.

(iii) The Examiner seeks to prematurely shift the burden of proof to Applicants, when the Examiner's own initial burden of proof has not yet been satisfied. Specifically, the Examiner is requiring applicants to prove that OP-1 would not be expected to exhibit the harmful renal effects of other anti-inflammatory agents, when it is the Examiner who bears the initial burden of showing why OP-1 should be considered as the exception amongst anti-inflammatory agents. MPEP 2142 imposes the initial burden on the examiner, and this burden has not been met.

CLAIM 61

As indicated in the preceding section, a reasonable expectation of success has not been established for claim 2, 53, and 58. Claim 61 is identical to claim 58 except that it further recites "wherein the agent is administered continuously during the period of acute renal failure" and "wherein the period of acute renal failure lasts from one to three weeks." Therefore, the failure to establish a reasonable expectation of success for the method of claim 58 also applies to the method of claim 61, thus rendering claim 61 nonobvious.

A failure to establish a *prima facie* case of obviousness for claim 61 also arises from the failure of the Examiner to establish a basis as to how the combination of cited references teaches or suggests all the elements of claim 61. In particular, the Examiner has not shown how the combination of cited references allegedly teaches (i) the treatment of a period of acute renal failure lasting from only one to three weeks; and (ii) the continuous administration of OP-1 during this one to three week period of acute renal failure.

Rather than specifically pointing out how these two elements are allegedly taught by the combination of references, the Examiner merely alleges that "the differences between the teachings of the references relied upon and the limitations of claims 60-65 would have been obvious absent any evidence that these differences are unexpected and unobvious" (page 3, lines

6-8 of the Office Action dated September 26, 2006). A single circular conclusory statement, however, is insufficient to satisfy the Examiner's burden of establishing a *prima facie* case of obviousness under MPEP § 706.02(j). The Examiner must specifically point out how all the elements of claim 61, including the two cited above, are allegedly taught by the combination of references, must point out how the references could be combined to achieve the claimed method, and must point out why one skilled in the art would have had a reasonable expectation of success in treating acute renal failure by administering the morphogen only during a period of one to three weeks. Since the Examiner has failed to show meet these three burdens, a *prima facie* case of obviousness has not been made.

CLAIM 64

As indicated in the preceding section, a reasonable expectation of success has not been established for claims 2, 53 and 58. Claim 64 is identical to claim 58 except that it further recites "wherein the mammal is afflicted with osteodystrophy." Therefore, the failure to establish a reasonable expectation of success for the method of claim 58 also applies to the method of claim 64, rendering claim 64 also nonobvious.

A failure to establish a *prima facie* case of obviousness also arises from the failure of the Examiner to establish a basis as to how the combination of cited references teaches or suggests the treatment of a subject afflicted with osteodystrophy as recited in claim 64.

The Examiner has not identified any teachings or suggestions in the combination of cited references for treating subjects who are additionally afflicted with osteodystrophy. Instead, the Examiner impermissibly tries to use the specification itself as one of the 103(a) references. The Examiner claims to use "the specification as a dictionary for [the] definition of subjects for treatment" (page 3, lines 20-21 of the Office Action dated September 21, 2006), and concludes that it would have been obvious to treat a subject afflicted with osteodystrophy.

But it is the combination of cited reference, and not the specification of the subject application, that must teach or suggest all the claim elements. This section of the specification states that "[i]n some number of cases, however, the subjects may present with other symptoms (e.g. osteodystrophy) for which morphogen treatment would be indicated." (page 12, lines 5-6).

Amended Appeal Brief dated September 20, 2007

Response to Notice of Non-Compliant Appeal Brief dated August 21, 2007

The Examiner cannot use the specification as a reference against itself. The suggestion or teaching to treat subjects afflicted with osteodystrophy must be found in the prior art. And the section of the specification cited by the Examiner is not providing any type of definition. It is showing embodiments of subjects that may be treated with OP-1.

The Examiner has failed to meet his burden of establishing why treatment of ARF patients additionally afflicted with osteodystrophy is allegedly taught by the prior art, and therefore has failed to make a *prima facie* case of obviousness under MPEP § 706.02(j).

CONCLUSION

In sum, the Examiner has not established a *prima facie* case of obviousness, either over Kelly in view of Kuberanpath and Lefer, on their own or in further view of Anderson and Brady, since he failed to show a reasonable expectation of success for using anti-inflammatory morphogens to improve renal function because anti-inflammatory agents were expected to do the opposite: to decrease renal function, i.e., the prior art teaches away from the present invention. Therefore, all independent claims are nonobvious over the cited references, including claims 2, 53, 58, 61 and 64. Furthermore, for claims 61 and 64, the Examiner has failed to show how the cited references teach or suggest all their claim elements.

Applicant believes no fee is due for the filing of this Appeal Brief. However, if fee is due, please charge our Deposit Account No. 18-1945, under Order No. JJJ-P01-514 from which the undersigned is authorized to draw.

Dated: September 20, 2007

Respectfully submitted,

By


Erika Takeuchi
Registration No.: 55,669
ROPES & GRAY LLP
1211 Avenue of the Americas
New York, NY 10036
(212) 596-9000
(212) 596-9090 (Fax)
Attorneys/Agents For Applicant

VIII. CLAIMS APPENDIX

The claims involved in the present appeal are shown below. Canceled claims are not shown. No claims are presently allowed.

2. A method of effecting an improvement in a standard marker of renal function in a mammal afflicted with acute renal failure, the method comprising administering to said mammal a therapeutically effective amount of an OP/BMP renal therapeutic agent comprising a polypeptide comprising a sequence at least 70% homologous to the C terminal seven-cysteine domain of human OP-1, the sequence of the C terminal seven-cysteine domain of human OP-1 being set forth at residues 330-431 of human OP-1, wherein said renal therapeutic agent:
 - (a) induces chondrogenesis in an ectopic bone assay; or
 - (b) prevents, inhibits, delays or alleviates loss of renal function resulting from acute renal failure in an animal model of acute renal failure;thereby effecting an improvement in a standard marker of renal function in the mammal afflicted with acute renal failure.
5. The method of claim 2 or 53, wherein said renal therapeutic agent comprises a polypeptide consisting of at least a C-terminal cysteine domain of a protein selected from the group consisting of a pro form, a mature form, and a soluble form of a polypeptide selected from the group consisting of OP-1, OP-2, OP-3, BMP2, BMP3, BMP4, BMP5, BMP6, and BMP9.
6. The method as in claim 5, wherein said renal therapeutic agent comprises a polypeptide consisting of at least a C-terminal cysteine domain of a protein selected from the group consisting of a pro form, a mature form, and a soluble form of human OP-1.
8. The method of claim 2, wherein said polypeptide has at least 75% homology with an amino

acid sequence of a seven-cysteine domain of human OP-1.

9. The method of claim 2, wherein said polypeptide has at least 80% homology with an amino acid sequence of a seven-cysteine domain of human OP-1.
11. The method of claim 53, wherein said polypeptide has at least 65% identity with an amino acid sequence of a seven-cysteine domain of human OP-1.
12. The method of claim 53, wherein said polypeptide has at least 70% identity with an amino acid sequence of a seven-cysteine domain of human OP-1.
14. The method of claim 2 or 53, wherein said renal therapeutic agent is selected from the group consisting of human osteogenic proteins and human bone morphogenic proteins.
15. The method of claim 2 or 53, wherein serial determination of BUN in said mammal indicates a rate of increase in BUN of at least 2 to 4 mmol/L/day (5 to 10 mg/dL/day).
16. The method of claim 2 or 53, wherein serial determination of BUN in said mammal indicates a rate of increase in BUN of at least 4 to 8 mmol/L/day (10 to 20 mg/dL/day).
17. The method of claim 2 or 53, wherein serial determination of serum creatinine in said mammal indicates a rate of increase in serum creatinine of at least 20 to 40 μ mol/L/day (0.25 to 0.5 mg/dL/day).
18. The method of claim 2 or 53, wherein serial determination of serum creatinine in said mammal indicates a rate of increase in serum creatinine of at least 40 to 80 μ mol/L/day (0.5 to 1.0 mg/dL/day).
19. The method of claim 2 or 53, wherein said mammal is afflicted with acute renal failure caused by a pre-renal cause, a post-renal cause, or an intrinsic renal cause.

20. The method of claim 19, wherein said mammal is afflicted with acute renal failure caused by a pre-renal cause selected from the group consisting of decreased cardiac output, hypovolemia, volume redistribution, and altered vascular resistance.
21. The method of claim 19, wherein said mammal is afflicted with acute renal failure caused by a post-renal cause selected from the group consisting of ureteral, pelvic and bladder obstructions.
22. The method of claim 19, wherein said mammal is afflicted with acute renal failure caused by an intrinsic renal cause selected from the group consisting of abnormalities of the vasculature, abnormalities of the glomeruli, acute interstitial nephritis, intratubular obstruction, renal artery occlusion and acute tubular necrosis.
23. The method of claim 2 or 53, wherein said mammal is a kidney transplant recipient.
24. The method of claim 2 or 53, wherein said mammal possesses only one kidney.
25. The method of claim 2 or 53, wherein said administration is oral.
26. The method of claim 2 or 53, wherein said administration is parenteral.
27. The method of claim 2 or 53, wherein said administration is intravenous.
28. The method of claim 2 or 53, wherein said administration is intraperitoneal.
29. The method of claim 2 or 53, wherein said administration is into the renal capsule.
30. The method of claim 26, wherein a stent has been implanted into said mammal for said administration.

31. The method of claim 30, wherein said stent is an intravenous stent.
32. The method of claim 30, wherein said stent is an intraperitoneal stent.
33. (The method of claim 30, wherein said stent is a renal intracapsular stent.
34. The method of claim 26, wherein said administration is by an implanted device.
35. The method of claim 2 or 53, wherein said administration is daily for a period of at least about one week.
36. The method of claim 2 or 53, wherein said administration is at least once a week for a period of at least about one month.
37. The method of claim 2 or 53, wherein said renal therapeutic agent is administered at a dosage of about 0.01-1000 $\mu\text{g}/\text{kg}$ body weight of said mammal.
38. The method of claim 37, wherein said renal therapeutic agent is administered at a dosage of about 0.1-100 $\mu\text{g}/\text{kg}$ body weight of said mammal.
53. A method of effecting an improvement in a standard marker of renal function in a mammal afflicted with acute renal failure, the method comprising administering to said mammal a therapeutically effective amount of an OP/BMP renal therapeutic agent comprising a polypeptide comprising a sequence at least 60% identical to the C terminal seven-cysteine domain of human OP-1, the sequence of the C terminal seven-cysteine domain of human OP-1 being set forth at residues 330-431 of human OP-1, wherein said renal therapeutic agent:
 - (a) induces chondrogenesis in an ectopic bone assay; or
 - (b) prevents, inhibits, delays or alleviates loss of renal function resulting from acute

renal failure in an animal model of acute renal failure;
thereby effecting an improvement in a standard marker of renal function in the mammal afflicted with acute renal failure.

54. The method of claim 53, wherein the standard marker of kidney function is a rate of increase in BUN levels, rate of increase in serum creatinine, static measurement of BUN, static measurement of serum creatinine, glomerular filtration rate (GFR), ratio of BUN/creatinine, serum concentration of sodium (Na⁺), urine/plasma ratio for creatinine, urine/plasma ratio for urea, urine osmolarity, or daily urine output.
55. The method of claim 2, wherein the standard marker of kidney function is a rate of increase in BUN levels, rate of increase in serum creatinine, static measurement of BUN, static measurement of serum creatinine, glomerular filtration rate (GFR), ratio of BUN/creatinine, serum concentration of sodium (Na⁺), urine/plasma ratio for creatinine, urine/plasma ratio for urea, urine osmolarity, or daily urine output.
56. The method of claim 2, wherein administration of the OP/BMP renal therapeutic agent delays the need for, or reduces the frequency of, dialysis treatments of the mammal afflicted with acute renal failure.
57. The method of claim 53, wherein administration of the OP/BMP renal therapeutic agent delays the need for, or reduces the frequency of, dialysis treatments of the mammal afflicted with acute renal failure.
58. A method of effecting an improvement in a standard marker of renal function in a mammal afflicted with acute renal failure, the acute renal failure being one arising from a pre-renal cause of acute renal failure, the method comprising administering to said mammal a therapeutically effective amount of an OP/BMP renal therapeutic agent comprising a polypeptide comprising a sequence at least 60% identical or 70%

homologous to the C terminal seven-cysteine domain of human OP-1, the sequence of the C terminal seven-cysteine domain of human OP-1 being set forth at residues 330-431 of human OP-1, wherein said renal therapeutic agent:

- (a) induces chondrogenesis in an ectopic bone assay; or
- (b) prevents, inhibits, delays or alleviates loss of renal function resulting from acute renal failure in an animal model of acute renal failure;

thereby effecting an improvement in a standard marker of renal function in the mammal afflicted with acute renal failure arising from a pre-renal cause of acute renal failure.

59. The method of claim 58, wherein the pre-renal cause of acute renal failure is selected from decreased cardiac output, hypovolemia, volume redistribution, and altered vascular resistance.
60. The method of claim 58, wherein the agent is administered-continuously during the period of acute renal failure.
61. A method of effecting an improvement in a standard marker of renal function in a mammal afflicted with acute renal failure, the acute renal failure being one arising from a pre-renal cause of acute renal failure, the method comprising administering to said mammal a therapeutically effective amount of an OP/BMP renal therapeutic agent comprising a polypeptide comprising a sequence at least 60% identical or 70% homologous to the C terminal seven-cysteine domain of human OP-1, the sequence of the C terminal seven-cysteine domain of human OP-1 being set forth at residues 330-431 of human OP-1, wherein said renal therapeutic agent:
 - (a) induces chondrogenesis in an ectopic bone assay; or
 - (b) prevents, inhibits, delays or alleviates loss of renal function resulting from acute renal failure in an animal model of acute renal failure;wherein the agent is administered continuously during the period of acute renal failure, wherein the period of acute renal failure lasts from one to three weeks,

thereby effecting an improvement in a standard marker of renal function in the mammal afflicted with acute renal failure arising from a pre-renal cause of acute renal failure.

62. The method of claim 58, wherein the acute renal failure is characterized by a deterioration of renal function over a period of a few days.
63. The method of claim 58, wherein serial determination of serum creatinine in said mammal indicates a rate of increase in serum creatinine exceeding 100 mg/dL/day.
64. A method of effecting an improvement in a standard marker of renal function in a mammal afflicted with acute renal failure, the acute renal failure being one arising from a pre-renal cause of acute renal failure, the method comprising administering to said mammal a therapeutically effective amount of an OP/BMP renal therapeutic agent comprising a polypeptide comprising a sequence at least 60% identical or 70% homologous to the C terminal seven-cysteine domain of human OP-1, the sequence of the C terminal seven-cysteine domain of human OP-1 being set forth at residues 330-431 of human OP-1, wherein said renal therapeutic agent:
 - (a) induces chondrogenesis in an ectopic bone assay; or
 - (b) prevents, inhibits, delays or alleviates loss of renal function resulting from acute renal failure in an animal model of acute renal failure;wherein the mammal is afflicted with osteodystrophy, thereby effecting an improvement in a standard marker of renal function in the mammal afflicted with acute renal failure arising from a pre-renal cause of acute renal failure.
65. The method of claim 58, wherein the mammal requires continuous hemodialysis sessions.

IX. EVIDENCE APPENDIX

No evidence pursuant to §§ 1.130, 1.131, or 1.132 or entered by or relied upon by the Examiner is being submitted. References previously cited by Appellant during prosecution and are relied upon to support this Appeal Brief are included as Exhibits D, F, I, K, L, and M.

X. RELATED PROCEEDINGS APPENDIX

No related proceedings are referenced in section II above. Therefore, copies of decisions in related proceedings do not exist; hence none are included.

EXHIBIT D

Increased expression of transforming growth factor- β in renal disease

Markus Ketteler, MD, Nancy A. Noble, PhD,
and Wayne A. Border, MD

University of Utah School of Medicine, Salt Lake City, Utah, USA

Transforming growth factor- β (TGF- β) is a multifunctional cytokine and a major regulator of tissue repair and extracellular matrix. Recent studies show that TGF- β overexpression in experimental and human kidney diseases leads to progressive glomerular and tubulointerstitial scarring and renal failure. New evidence suggests that angiotensin-converting enzyme inhibitors and a low-protein diet may slow the progression of chronic kidney diseases in part by suppressing TGF- β overexpression. New therapies may prevent progressive fibrosis in chronic kidney disease by suppressing the action of TGF- β .

Current Opinion in Nephrology and Hypertension 1994, 3:446-452

Chronic progressive kidney diseases are characterized by the accumulation of fibrotic tissue in the glomerulus and in the tubulointerstitium [1,2]. This process leads to progressive loss of kidney function and finally to kidney failure, regardless of the primary disorder [1,2]. Transforming growth factor- β (TGF- β) has been identified as an important mediator of chronic fibrosis in several disease states [2-4]. The role of TGF- β in fibrogenesis is beneficial in wound repair but deleterious in chronic fibrotic diseases. This review characterizes briefly the biology of TGF- β , summarizes recent experimental data on its role in kidney diseases, and outlines potential therapeutic strategies to target TGF overexpression in disease.

Molecular biology

Transforming growth factor- β belongs to a family of multifunctional cytokines that are dimeric proteins with a molecular weight of approximately 25 kD [5,6]. Three isoforms (TGF- β_1 , - β_2 , and - β_3) located on different chromosomes have been identi-

fied in mammalian species [5,6]. Cleavage from a larger precursor molecule is necessary for TGF- β to exert biologic activity in tissues [5,6]. Three different TGF- β receptors have been identified. Recent data suggest that the type I and type II receptors are involved in signal transduction, whereas the type III receptor may serve as a TGF- β -binding reservoir [6].

Transforming growth factor- β plays a major biologic role in the regulation of cellular proliferation and growth, extracellular matrix synthesis, chemoattraction, angiogenesis, and in the induction of cytokines or their receptors [2-4,5,6]. TGF- β can induce its own synthesis by cells exposed to it [7,8]. This property of autoinduction may be central to its continued overexpression in chronic fibrosis. TGF- β profoundly alters three processes involved in extracellular matrix deposition: 1) it induces synthesis of numerous extracellular matrix proteins; 2) it decreases matrix degradation by down-regulation of proteases and induction of protease inhibitors; and 3) it enhances the expression of integrins on the cell surface, which facilitates the deposition of matrix [5]. These properties

Abbreviations

ACE—angiotensin-converting enzyme; ATS—antithymocyte antibodies; TGF- β —transforming growth factor- β .

are of major importance in normal wound healing. When normal repair is complete TGF- β expression returns to normal. In contrast, chronic fibrosis is characterized by continuous TGF- β overexpression [9**].

Acute renal injury

The basic studies on the role of TGF- β in glomerular injury and repair were performed in a rat model of immune-mediated, mesangiproliferative glomerulonephritis induced by antithymocyte antibodies (ATS) [10-14]. ATS recognizes a thy-1-like antigen on the mesangial cell surface and induces complement-dependent mesangial cell lysis [11]. The subsequent mesangial cell proliferation and glomerular extracellular matrix accumulation resemble the changes observed in mesangiproliferative glomerulonephritis in humans except these are self-limiting and normalize within several months.

Using this model, we demonstrated that TGF- β expression increases in parallel with extracellular matrix deposition [10]. Administration of TGF- β -neutralizing antibodies *in vivo* prevented extra-

cellular matrix accumulation thus identifying TGF- β as a key mediator of fibrosis in this model [12].

The fibrogenic effects of TGF- β were shown to be due to three actions. First, TGF- β induced the synthesis of the extracellular matrix components that accumulate in glomerulosclerosis [10,12]. Second, TGF- β decreased the action of the plasmin protease system, which is thought to be important in extracellular matrix turnover [13]. TGF- β decreases the action of plasmin by depressing synthesis of the enzyme needed to generate plasmin and plasminogen activator, and simultaneously increases synthesis of its inhibitor plasminogen activator inhibitor type 1 [13]. Third, synthesis of $\beta 1$ integrins, which play an important role in extracellular matrix assembly, was increased in ATS-induced glomerulonephritis following elevated TGF- β expression [14]. Interestingly, the addition of TGF- β to normal glomeruli in culture also increased the synthesis of matrix components, inhibited the plasmin protease system, and upregulated the expression of $\beta 1$ integrins on the cells' surface [10,13,14].

Increased TGF- β expression has also been reported in mesangiproliferative glomerulonephritis induced by Habu snake venom, in animal models

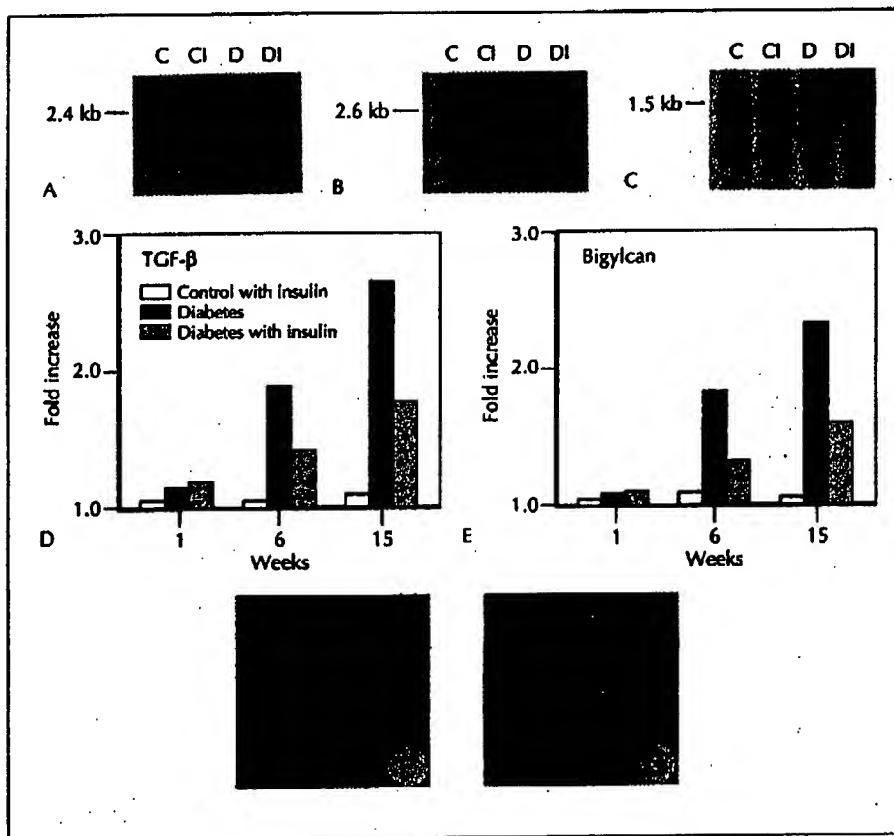


Fig. 1. Glomerular transforming growth factor- β (TGF- β) expression in streptozocin-induced diabetes in the rat. TGF- β (A), biglycan (B), and the control enzyme glyceraldehyde-3-phosphate dehydrogenase (C) messenger RNA expression in glomeruli as detected by Northern blot hybridization at 15 weeks after induction of diabetes. Relative increases of TGF- β (D) and biglycan (E) messenger RNA in the time course of diabetes are diagrammed. Glomerular TGF- β protein expression is shown by immunofluorescence in control rats (F) versus in untreated rats with diabetes 15 weeks after induction of diabetes (G). C—control rats; CI—control rats treated with insulin; D—rats with diabetes; DI—rats with diabetes treated with insulin. (From Yamamoto et al. [24**]; with permission.)

of crescentic nephritis induced by antiglomerular basement membrane antibodies, and in acute tubulointerstitial injury (puromycin aminonucleoside nephrosis) [15-17]. In all cases, TGF- β expression was accompanied by increased matrix protein deposition. In crescentic nephritis, urinary TGF- β excretion was correlated with the degree of glomerular sclerosis, suggesting that urinary TGF- β might be a useful indicator in progressive renal disease [18]. Recently, TGF- β upregulation was observed following unilateral ureteral ligation in a rat model of obstructive nephropathy [19]. Together, these studies show that the kidney responds to injury with increased TGF- β expression that, in turn, causes fibrosis.

Chronic renal disease

Early studies in acute models of renal injury pointed to a key role of TGF- β induction in renal disease. The question of whether or not sustained TGF- β expression played a role in chronic progressive kidney disease, however, remained unaddressed. We answered this question by the induction of chronic renal disease using two injections of ATS [9^{**}]. Two consecutive injections of ATS given 1 week apart led to progressive glomerular and tubulointerstitial sclerosis accompanied by increasing proteinuria [9^{**}]. In the one-injection ATS model TGF- β expression normalized after several weeks, but rats given two ATS injections showed progressively increased glomerular TGF- β expression that with time involved the tubulointerstitium [9^{**},10]. Histologic changes closely resembled those seen during the development of severe renal fibrosis in humans [9^{**}]. Both TGF- β expression and TGF- β receptors were increased in another model of chronic renal disease induced by doxorubicin hydrochloride (adriamycin model) [20].

Repeated injections of puromycin aminonucleoside also led to chronic renal fibrosis [21]. When fibrosis was apparent in this disease model, glomerular TGF- β messenger RNA expression was 15 times elevated compared with control animals [21]. Platelet-derived growth factor and basic fibroblast growth factor expression were increased, but to a lesser extent [21]. Because TGF- β is a potent inducer of platelet-derived growth factor protein and receptor expression, it likely enhances the effects of other growth factors in chronic progressive kidney disease [22].

Insulin-dependent diabetes mellitus can be induced in rats by a bolus injection of streptozocin [23] and the diabetic complications of retinopathy and nephropathy develop within months. In humans, diabetic nephropathy is the leading cause of renal failure and is characterized by progressive diffuse glomerulosclerosis. We hypothesized that TGF- β is involved in this process. Progressive increases in TGF- β messenger RNA expression were seen in glomeruli obtained from diabetic rats as early as 6 weeks after induction of disease (Fig. 1) [24^{**}]. This increase paralleled deposition of fibronectin and tenascin, two important components of extracellular matrix. It was also recently reported that glomerular collagen synthesis is upregulated by TGF- β in diabetic rats [25].

An important experiment confirmed the ability of TGF- β alone to cause glomerulosclerosis. Isaka *et al.* [26^{**}] injected liposomes containing TGF- β complementary DNA into the left renal artery of otherwise normal rats. This was followed by increased expression of the TGF- β protein and severe glomerulosclerosis within 7 days. The right kidney was completely unaffected by this procedure, which clearly demonstrates that TGF- β alone is a potent inducer of extracellular matrix accumulation in glomeruli.

Table 1. Elevated transforming growth factor- β expression in experimental renal disease

Study	Model	Disease
Yamamoto <i>et al.</i> [9 ^{**}]	Animal (chronic)	Glomerulosclerosis (induced by repeated antithymocyte antibody injections)
Okuda <i>et al.</i> [10]	Animal (acute)	Antithymocyte antibody-induced glomerulonephritis
Barnes and Abboud [15]	Animal (acute)	Mesangioproliferative glomerulonephritis (Habu snake venom)
Coimbra <i>et al.</i> [16]	Animal (acute)	Antiglomerular basement membrane nephritis
Jones <i>et al.</i> [17]	Animal (acute)	Puromycin aminonucleoside nephrosis
Kaneto <i>et al.</i> [19]	Animal (acute)	Unilateral ureteral ligation
Tamaki <i>et al.</i> [20]	Animal (chronic)	Glomerulosclerosis (induced by adriamycin injection)
Nakamura <i>et al.</i> [21]	Animal (chronic)	Glomerulosclerosis (induced by repeated puromycin aminonucleoside injections)
Yamamoto <i>et al.</i> [24 ^{**}]	Animal (chronic)	Diabetic nephropathy
Junaid <i>et al.</i> [32]	Animal (chronic)	Remnant kidney (subtotal nephrectomy)

All of these studies show that TGF- β is a major mediator of chronic glomerular and tubulointerstitial fibrosis in progressive kidney diseases. Investigations using numerous other models of organ fibrosis in liver, lung, skin, arteries, and central nervous system confirm that TGF- β plays a role in chronic fibrosis [2,3].

Human kidney disease

Recent reports show that the experimental data presented thus far are relevant to progressive kidney disease in humans. Kidney biopsies from patients with overt diabetic nephropathy showed significantly increased glomerular TGF- β expression compared both with normal kidney tissue and with material obtained from patients with minimal change disease and thin basement membrane disease; these diseases do not progress to sclerosis [24**]. The quantity of staining closely correlated with the severity of glomerulosclerosis. Table 1 lists renal disease models in which elevated TGF- β expression has been found.

The renin-angiotensin system and transforming growth factor- β

Angiotensin II is thought to damage kidneys by increasing glomerular hydrostatic capillary and sys-

temic blood pressure [27]. The beneficial effects of angiotensin-converting enzyme (ACE) inhibitors in slowing disease progression support this point of view [28]. Recent studies on the interaction of the renin-angiotensin system and TGF- β suggest that another pathway exists.

Gibbons *et al.* [29] showed that angiotensin II induces TGF- β messenger RNA expression in cultured vascular smooth muscle cells. Wolf *et al.* [30] confirmed these findings in proximal tubular cells *in vitro*, and both studies demonstrated that angiotensin II-induced TGF- β expression leads to hypertrophic growth. Using cultured rat mesangial cells, we recently reported that angiotensin II stimulates TGF- β expression and that TGF- β then induces increased synthesis of matrix proteins [31**]. *In vivo* subcutaneous infusion of angiotensin II led to increased glomerular TGF- β gene expression within 1 week. This increase was associated with the induction of collagen type I messenger RNA and glomerular matrix accumulation [31**]. Interestingly, in unilateral ureteral ligation and in the remnant kidney model in the rat, TGF- β messenger RNA expression was significantly blunted by *in vivo* administration of an ACE inhibitor or an angiotensin II receptor antagonist, respectively [19,32]. These data suggest that angiotensin II directly induces TGF- β , which in turn induces fibrotic changes.

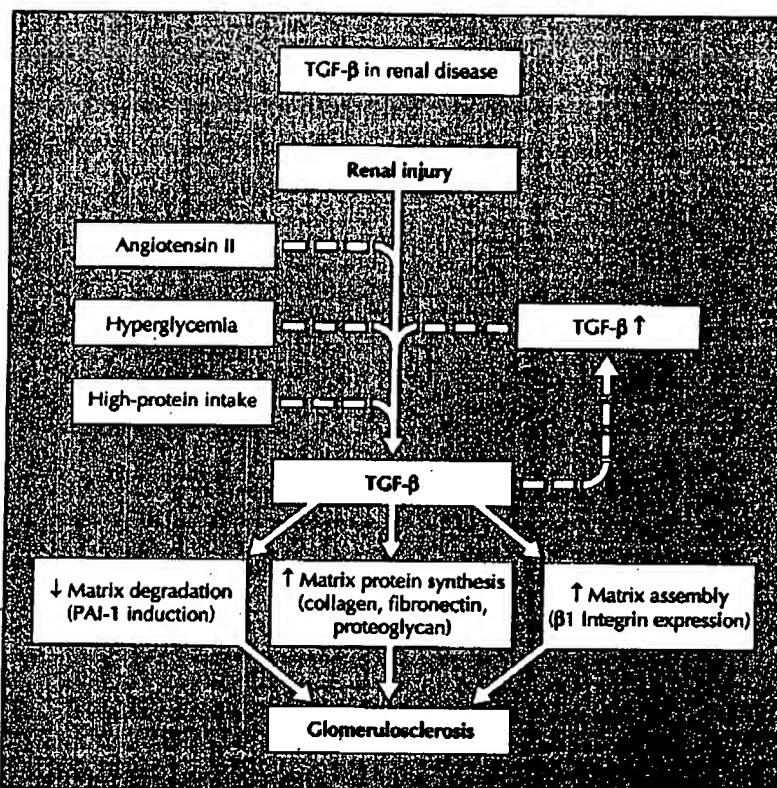


Fig. 2. The role of transforming growth factor- β (TGF- β) overexpression in the pathogenesis of glomerulosclerosis and potential targets of treatment. PAI—plasminogen activator inhibitor.

Therapeutic strategies targeting transforming growth factor- β overexpression

Three clear strategies exist to decrease TGF- β expression *in vivo*: inhibition of angiotensin II, low-protein diet, and direct antagonists of TGF- β . In addition to influencing the hemodynamic effects of angiotensin II, ACE inhibitors and angiotensin II receptor antagonists may also inhibit the angiotensin II-induced cascade of both increased TGF- β expression and increased matrix protein synthesis and deposition. It will be interesting to determine whether or not these drugs lead to a reduction in TGF- β expression *in vivo*.

Another treatment slowing the progression of some renal diseases is a low-protein diet, which decreases glomerular hyperfiltration and reduces plasma renin activity [33-35]. We showed that dietary protein restriction downregulated TGF- β expression in ATS-induced glomerulonephritis [36]. A decrease in the activity of the renin-angiotensin system may have contributed to this effect. Recent data from our laboratory suggest that restriction of the amino acid L-arginine may be the key factor in low-protein diet because low dietary L-arginine also downregulates TGF- β expression in this model, even when total dietary protein intake is normal [37]. Metabolites of L-arginine are involved in tissue injury (nitric oxide), cell proliferation (polyamines), and collagen synthesis (L-proline), processes ongoing in many renal diseases.

A number of antagonists of TGF- β are possible. Soluble receptors, antisense oligonucleotides, and *humanized* antibodies are three possibilities. Another potential therapeutic antagonist is the proteoglycan decorin, which binds and neutralizes TGF- β *in vitro* [38]. Decorin is a normal extracellular matrix component that can be manufactured in large quantities using recombinant technology. In ATS-induced glomerulonephritis, the *in vivo* administration of decorin significantly blunted extracellular matrix accumulation within the glomerulus and was as potent as an injection of TGF- β neutralizing antibody [39].

The mechanism of decorin's antagonism of TGF- β and its efficacy in progressive kidney fibrosis are currently being studied. A study of the bleomycin model of pulmonary lung fibrosis in the rat found decreased decorin expression in the initial phases of the disease followed by increased expression during the reparative phase [40]. It is possible that decorin plays a role in the termination of normal wound repair by blocking the TGF- β effects and by interrupting TGF- β autoinduction. Progressive

fibrosis might then represent a state of decorin deficiency.

Conclusions

The experimental and human studies reviewed show that TGF- β plays a causative role in glomerular and tubulointerstitial fibrosis; potential mechanisms are summarized in Figure 2. Antagonists of the action of TGF- β are therefore very promising therapeutic agents. ACE inhibitors and low-protein diets may slow the progressive course of some kidney diseases by suppressing TGF- β expression. The TGF- β binding proteoglycan decorin may become an antifibrotic drug for the treatment of chronic progressive kidney failure.

Acknowledgment

The writing of this review and the original work by the authors is supported by grant DK 43609 from the National Institute of Diabetes and Digestive Kidney Diseases.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- Of special interest
- ++ Of outstanding interest

1. Klahr S, Schreiner G, Ichikawa I: The Progression of Renal Disease. *N Engl J Med* 1988, 318:1657-1666.
2. Border WA, Ruoslahti E: Transforming Growth Factor- β in Disease: The Dark Side of Tissue Repair. *J Clin Invest* 1992, 90:1-7.
3. Roberts AB, Sporn MB: Physiological Action and Clinical Applications of Transforming Growth Factor- β (TGF- β). *Growth Factors* 1993, 8:1-9.
4. Roberts AB, McCune BK, Sporn MB: TGF- β : Regulation of Extracellular Matrix. *Kidney Int* 1992, 41:557-559.
5. Roberts AB, Sporn MB: The Transforming Growth Factor- β s. In *Handbook of Experimental Pharmacology: Peptide Growth Factors and Their Receptors: 1*. Edited by Sporn MB, Roberts AB. Berlin, Heidelberg, New York: Springer Verlag; 1992:419-472.
- A comprehensive review on the basic biology of TGF- β .
6. Miyazono K, Ichijo H, Heldin CH: Transforming Growth Factor- β : Latent Forms, Binding and Receptors. *Growth Factors* 1993, 8:11-22.
7. Kim SJ, Angel P, Lafyatis R, Hattori K, Kim KY, Sporn MB, Karin M, Roberts AB: Autoinduction of Transforming Growth Factor- β_1 Is Mediated by the AP-1 Complex. *Mol Cell Biol* 1990, 10:1492-1497.

8. Kaname S, Uchida S, Ogata E, Kurokawa K: Autocrine Secretion of Transforming Growth Factor- β in Cultured Rat Mesangial Cells. *Kidney Int* 1992, 42:1319-1327.

9. Yamamoto T, Noble NA, Miller DA, Border WA: Sustained Expression of TGF- β_1 Underlies Development of Progressive Kidney Fibrosis. *Kidney Int* 1994, 45:916-927.

This study presents conclusive data demonstrating that TGF- β overexpression is strongly associated with the development of progressive glomerular and tubulointerstitial sclerosis. This animal model closely resembles morphologic changes of chronic glomerulonephritis in humans.

10. Okuda S, Languino LR, Ruoslahti E, Border WA: Elevated Expression of Transforming Growth Factor- β and Proteoglycan Production in Experimental Glomerulonephritis. *J Clin Invest* 1990, 86:453-462.

11. Yamamoto Y, Wilson CB: Quantitative and Qualitative Studies of Antibody-Induced Mesangial Cell Damage in the Rat. *Kidney Int* 1987, 32:514-525.

12. Border WA, Okuda S, Languino L, Sporn MB, Ruoslahti E: Suppression of Experimental Glomerulonephritis by Antiserum Against Transforming Growth Factor- β_1 . *Nature* 1990, 346:371-374.

13. Tomooka S, Border WA, Marshall BC, Noble NA: Glomerular Matrix Accumulation Is Linked to Inhibition of the Plasmin Protease System. *Kidney Int* 1992, 42:1462-1469.

14. Kagami S, Border WA, Ruoslahti E, Noble NA: Coordinated Expression of β_1 Integrins and Transforming Growth Factor- β -Induced Matrix Proteins in Glomerulonephritis. *Lab Invest* 1993, 69:68-76.

15. Barnes JL, Abboud HE: Temporal Expression of Autocrine Growth Factors Corresponds to Morphological Features of Mesangial Proliferation in Habu Snake Venom-Induced Glomerulonephritis. *Am J Pathol* 1993, 143:1366-1376.

16. Coimbra T, Wiggins R, Noh JW, Merritt S, Phan SH: Transforming Growth Factor- β Production in Anti-Glomerular Basement Membrane Disease in the Rabbit. *Am J Pathol* 1991, 138:223-224.

17. Jones C, Buch S, Post M, McCulloch L, Liu E, Eddy A: Renal Extracellular Matrix Accumulation in Acute Puromycin Aminonucleoside Nephrosis. *Am J Pathol* 1992, 141:1381-1396.

18. Noh JW, Wiggins R, Phan SH: Urine Transforming Growth Factor- β Activity Is Related to the Degree of Scarring in Crescentic Nephritis in the Rabbit. *Nephron* 1993, 63:73-78.

19. Kaneto H, Morrissey J, Klahr S: Increased Expression of TGF- β_1 mRNA in the Obstructed Kidney of Rats With Unilateral Ureteral Ligation. *Kidney Int* 1993, 44:313-321.

20. Tamaki K, Okuda S, Ando T, Iwamoto T, Nakayama M, Fujishima M: TGF- β_1 in Glomerulosclerosis and Interstitial Fibrosis of Adriamycin Nephropathy. *Kidney Int* 1994, 45:525-536.

21. Nakamura T, Ebihara E, Fukui M, Oeada S, Nagaoka I, Horikoshi S, Tomino Y, Koide H: Messenger RNA Expression for Growth Factors in Glomeruli From Focal Glomerular Sclerosis. *Clin Immunol Immunopathol* 1993, 66:33-42.

22. Haberstroh U, Zahner G, Disser M, Thaiss F, Wolf G, Stahl RAK: TGF- β Stimulates Rat Mesangial Cell Proliferation in Culture: Role of PDGF β -Receptor Expression. *Am J Physiol* 1993, 264:F199-F205.

23. Mauer SM, Steffes MW, Michael AF, Brown DM: Studies of Diabetic Nephropathy in Animals and in Man. *Diabetes* 1976, 25:850-857.

24. Yamamoto T, Nakamura T, Noble NA, Ruoslahti E, Border WA: Expression of Transforming Growth Factor β Is Elevated in Human and Experimental Diabetic Nephropathy. *Proc Natl Acad Sci U S A* 1993, 90:1814-1818.

This is, to date, the first demonstration of TGF- β overexpression as a likely cause of glomerulosclerosis in diabetic nephropathy. Immunofluorescence microscopy is used for detection of TGF- β in sclerotic glomeruli obtained from patients with diabetic nephropathy. These data are compared with TGF- β messenger RNA and protein expression in the time course of streptozocin-induced diabetes in the rat.

25. Bollineni JS, Reddi AS: Transforming Growth Factor β_1 Enhances Glomerular Collagen Synthesis in Diabetic Rats. *Diabetes* 1993, 42:1673-1677.

26. Isaka Y, Fujiwara Y, Ueda N, Kaneda Y, Kamada T, Imai E: Glomerulosclerosis by *In Vivo* Transfection of Transforming Growth Factor β and Platelet-Derived Growth Factor Gene Into the Rat Kidney. *J Clin Invest* 1993, 92:2597-2601.

This study proves the assumption that selective TGF- β overexpression in glomeruli causes glomerulosclerosis. Liposomes containing TGF- β complementary DNA are injected into the left renal artery of otherwise normal rats. TGF- β protein is expressed locally and significant extracellular matrix accumulation follows in the left kidney. The right kidney remains completely unaffected. *In vivo* transfection represents an efficient and advanced technique to investigate cytokine effects.

27. Miller PL, Rennke HG, Meyer TW: Glomerular Hypertrophy Accelerates Hypertensive Glomerular Injury in Rats. *Am J Physiol* 1991, 261:F459-F465.

28. Brunner HR: ACE Inhibitors in Renal Disease. *Kidney Int* 1992, 42:463-479.

29. Gibbons GH, Pratt RE, Dzau VJ: Vascular Smooth Muscle Cell Hypertrophy vs Hyperplasia. *J Clin Invest* 1992, 90:456-461.

30. Wolf G, Muller E, Stahl RAK, Ziyadeh FN: Angiotensin II-Induced Hypertrophy of Cultured Murine Proximal Tubular Cells Is Mediated by Endogenous Transforming Growth Factor β . *J Clin Invest* 1993, 92:1366-1372.

31. Kagami S, Border WA, Miller DA, Noble NA: Angiotensin II Stimulates Extracellular Matrix Protein Synthesis Through Induction of Transforming Growth Factor- β Expression in Rat Glomerular Mesangial Cells. *J Clin Invest* 1994, 96:2431-2437.

Presents *in vitro* and *in vivo* data demonstrating that angiotensin II may directly lead to extracellular matrix accumulation, and thereby to expression and activation of latent TGF- β .

32. Junaid A, Rosenberg ME, Hostetter TH: Interaction of Angiotensin II (AII) and Transforming Growth Factor Beta (TGF- β) in the Remnant Kidney [Abstract]. *J Am Soc Nephrol* 1993, 4:772.

33. Brenner BM, Meyer TW, Hostetter TH: Dietary Protein Intake and the Progressive Nature of Kidney Disease: The Role of Hemodynamically Mediated Glomerular Sclerosis in Aging, Renal Ablation and Intrinsic Renal Disease. *N Engl J Med* 1982, 307:652-659.

34. Rosman JB, Meijer S, Sluiter WJ, Ter Wee PM, Piers-Becht TPM, Donker AJM: Prospective Randomised Trial of Early Dietary Protein Restriction in Chronic Renal Failure. *Lancet* 1984, ii:1291-1296.

35. Paller MS, Hostetter TH: Dietary Protein Increases Plasma Renin and Reduces Pressor Reactivity to Angiotensin II. *Am J Physiol* 1986, 251:F34-F39.

36. Okuda S, Nakamura T, Yamamoto T, Ruoslahti E, Border WA: Dietary Protein Restriction Rapidly Reduces Transforming Growth Factor β_1 Expression in Experimental Glomerulonephritis. *Proc Natl Acad Sci U S A* 1991, 88:9765-9769.

37. Noble NA, Narita I, Ketteler M, Border WA: L-Arginine Dependency of Glomerular Cell Repair in Experimental Glomerulonephritis [Abstract]. *J Am Soc Nephrol* 1993, 4:624.

38. Yamaguchi Y, Mann DM, Ruoslahti E: Negative Regulation of Transforming Growth Factor β by the Proteoglycan Decorin. *Nature* 1990, 346:281-284.

39. Border WA, Noble NA, Yamamoto T, Harper JR, Yamaguchi Y, Pierschbacher MD, Ruoslahti E: Natural Inhibitor of Transforming Growth Factor β Protects Against Scarring in Experimental Kidney Disease. *Nature* 1992, 360:361-364.

40. Westergren-Thorsson G, Hemminki S, Samstrand B, Olkberg A, Heinegard D, Malmstroem A: Altered Expression of Small Proteoglycans, Collagen and Transforming Growth Factor β_1 in Developing Bleomycin-Induced Pulmonary Fibrosis in Rats. *J Clin Invest* 1993, 92:632-637.

Wayne A. Border, MD, Division of Nephrology, University of Utah, 50 North Medical Drive, Salt Lake City, UT 84132, USA.

EXHIBIT F

Transforming growth factor- β and the pathogenesis of glomerular diseases

Wayne A. Border, MD

University of Utah School of Medicine, Salt Lake City, Utah, USA

Transforming growth factor- β (TGF- β) is a cytokine that is important in embryogenesis, development, carcinogenesis, and tissue repair. TGF- β is unique among cytokines in its widespread actions on the regulation of extracellular matrix. In a model of acute mesangial proliferative glomerulonephritis, it was shown that overproduction of TGF- β is the cause of pathologic matrix accumulation in the nephritic glomeruli. TGF- β acted to increase matrix production, inhibit matrix degradation, and modulate matrix receptors in the glomerulonephritic rats. Elevated expression of TGF- β was also found in other experimental glomerular diseases, including diabetic nephropathy. Studies of humans with glomerulonephritis and diabetic nephropathy also strongly implicate TGF- β in the pathogenesis of glomerular matrix build-up. Recently, the proteoglycan decorin was shown to neutralize TGF- β . When injected into glomerulonephritic rats, decorin markedly suppressed pathologic matrix deposition in the glomeruli. Thus, decorin offers hope as a treatment for progressive kidney diseases caused by the overproduction of TGF- β .

Current Opinion in Nephrology and Hypertension 1994, 3:54-58

Extracellular matrix is composed of a mixture of interacting glycoproteins, collagens, and proteoglycans. Cells attach directly to the matrix by specific receptors, called *integrins*, that connect via the cytoskeleton to the nucleus, providing a direct line of communication between the cell and the outside world. Cell attachment to matrix is essential for differentiation and the formation of specific tissues and is also a key event in carcinogenesis and tissue repair. Recent evidence shows that essential biologic events, such as embryogenesis, development, and tissue repair, are regulated by the actions of peptide factors called *cytokines* (or growth factors). Transforming growth factor- β (TGF- β) is a prototypical cytokine, and one of the major actions of TGF- β is regulation of extracellular matrix deposition. TGF- β is unique among cytokines in its actions to: 1) stimulate the synthesis of matrix proteins by cells, 2) inhibit the proteases that degrade matrix, and 3) modulate integrins on the cell surface to allow greater binding to matrix. These three actions strongly promote deposition and accumulation of matrix in tissues. Excessive deposition of extracellular matrix is the central feature of fibrotic diseases, such as glomerulonephritis, di-

abetic nephropathy, cirrhosis, and pulmonary fibrosis, that destroy organ function. A growing body of evidence strongly implicates TGF- β as the cause of these fibrotic diseases [1,2,3]. Abboud [4] has provided an excellent overview of the action of cytokines, including TGF- β , in glomerulonephritis.

Transforming growth factor- β and cultured glomerular cells

Previously, TGF- β was shown to stimulate the production of proteoglycans by cultured rat mesangial cells, whereas other cytokines (platelet-derived growth factor [PDGF], interleukin-1, and tumor necrosis factor) had no effect on matrix synthesis [5]. In a new report, this work was extended to rat glomerular (visceral) epithelial cells [6]. TGF- β was found to induce the production of fibronectin, type IV collagen, laminin, and biglycan. Of interest, TGF- β did not alter the synthesis of heparan sulfate, another component of the glomerular basement membrane. As with the mesangial cells,

Abbreviations

PA—plasminogen activator; PAI—plasminogen activator inhibitor;
PDGF—platelet-derived growth factor; TGF- β —transforming growth factor- β .

TGF- β did not stimulate cell proliferation. These results indicate that TGF- β , released in the glomerulus following injury, can cause both cell types to increase production of extracellular matrix. In a related study, TGF- β was found to increase the production of proteoglycans by rabbit renal proximal tubule cells and to increase the structural integrity of the cytoskeleton [7]. These effects may be important in the regeneration of the tubule following injury.

All cells, except for contrived mutants, synthesize and release TGF- β . Mesangial cells in culture express a 2.5-kb TGF- β mRNA and constitutively secrete a substantial amount of TGF- β , mostly in the latent form [8]. TGF- β is proteolytically processed into a 25-kD mature homodimer that is secreted noncovalently bound to a portion of the precursor protein that confers biologic latency. Either acidic conditions or proteases, *eg*, plasmin, can dissociate the latency peptide and release active TGF- β . How TGF- β is activated *in vivo* is unknown. PDGF was found to not alter TGF- β expression, however, phorbol ester did stimulate mesangial cells to express TGF- β mRNA. Thus, mesangial cells were shown to produce TGF- β in a manner implicating TGF- β as an autocrine factor that is biologically active in these cells [8].

In wound repair, TGF- β and PDGF are companion cytokines with quite distinct actions. Both are released by platelets at sites of injury such as in areas of mesangial cell lysis following administration of antithymocyte serum, as employed in a popular experimental model of mesangial proliferative glomerulonephritis [9]. Under standard conditions TGF- β does not stimulate mesangial cell proliferation and has unique actions on extracellular matrix, as already described [5,10]. In contrast, PDGF is a potent mitogen for mesangial cells but does not affect matrix synthesis [5,11]. Johnson *et al.* [9] emphasized the concept that PDGF-induced mesangial cell proliferation is a key component in mesangial matrix accumulation. Although dividing cells do produce matrix and an increased number of cells necessitates more matrix, it is clear that TGF- β is capable of increasing matrix production without cell proliferation [5]. Furthermore, TGF- β may regulate PDGF production by mesangial cells. TGF- β was shown to increase expression of mRNAs of both PDGF B chain and PDGF β -receptor [12]. The increased PDGF expression was associated with increased density of cell surface PDGF receptors and stimulation of mesangial cell proliferation. This report also summarized evidence from other cell lines in which PDGF is also regulated in a complex way by TGF- β [12]. One therapeutic implication of this work is that blocking TGF- β may simultaneously prevent matrix accumulation and excessive cell proliferation in glomerulonephritis.

Mesangial proliferative glomerulonephritis

Transforming growth factor- β has been shown to strongly induce matrix protein synthesis in cultured

glomerular cells and, *in vivo*, in acute mesangial proliferative glomerulonephritis induced by injection of antithymocyte serum [5,6,13]. A particularly powerful tool for investigating this model is the ability to acutely isolate and culture nephritic glomeruli in order to perform molecular studies. The use of these techniques in glomerular disease was recently reviewed [14]. By day 4 of glomerulonephritis, there was a substantial increase in synthesis of proteoglycans and fibronectin by the nephritic glomeruli. Simultaneously, there was increased deposition of matrix in the mesangial areas. The increased matrix production correlated with increased expression of TGF- β mRNA and protein in the nephritic glomeruli. That TGF- β was causal in stimulating the matrix production was shown by the ability of TGF- β antibody to suppress matrix synthesis when added to nephritic glomeruli in culture. In a subsequent study, injection of TGF- β antibody into nephritic rats dramatically suppressed the expected increase in matrix production by the nephritic glomeruli and prevented the build-up of pathologic matrix, which is characteristic of the disease [15].

The second action of TGF- β to inhibit matrix degradation was also investigated in this model [16]. One of the proteases strongly influenced by TGF- β is the plasminogen activator/plasmin system. Plasmin is a potent protease that is best known for its activity against fibrin, but plasmin is also capable of degrading most matrix proteins and probably plays an important role in normal matrix turnover. Plasmin generation is regulated by the interaction of plasminogen activators (PAs) and plasminogen activator inhibitors (PAIs). It was found that PA activity was markedly reduced and PAI synthesis dramatically increased when TGF- β was added to normal glomeruli in culture. In the glomerulonephritic model, it was shown that prior to matrix accumulation there were striking changes in the PA/PAI system that would favor matrix deposition [16]. PA activity was dramatically decreased and PAI synthesis increased by day 3 of disease, and both returned toward normal by day 7. The increased synthesis of PAI was reflected in increased PAI deposition into the glomerular matrix, where it acts to block plasmin generation. These findings indicate that the components necessary for blocking matrix degradation by plasmin are in place early in the disease process, prior to histologic evidence of matrix accumulation. Furthermore, a causal role for TGF- β in regulating these changes was demonstrated by showing that *in vivo* administration of TGF- β antibody blocked the disease-induced deposition of PAI in the glomerular matrix.

The third action of TGF- β influencing matrix deposition is the modulation of integrins on the cell surface that mediate cell matrix contact. Integrins constitute a family of heterodimeric glycoproteins consisting of noncovalently associated α and β subunits. The synthetic profiles of integrins during the course of disease were investigated in the acute glomerulonephritis model [17]. By day 7 of disease, there was a marked increase in

synthesis and expression of the classic fibronectin receptor $\alpha_5\beta_1$, which was paralleled by a build-up of fibronectin in the expanding mesangial matrix. Other integrin receptors not involved in matrix deposition showed no change or actually decreased during the course of the disease, indicating the physiological significance of the increase in the fibronectin receptor. This study provides the missing piece of the mechanistic puzzle concerning the actions of TGF- β . Thus, both increased synthesis and decreased degradation of matrix coupled with increases in the number of integrin receptors on glomerular cells contribute to the deposition of matrix components and the accumulation of pathologic matrix following glomerular injury.

Crescentic glomerulonephritis

In previous studies, Coimbra *et al.* [18] demonstrated increased TGF- β production in glomeruli isolated from rabbits with anti-glomerular basement membrane crescentic glomerulonephritis. In this model, the kidney develops rapid cortical fibrosis due to induction of interstitial collagen production immediately following glomerular injury [19]. In a new report, this group measured TGF- β levels in urine from the nephritic animals [20]. TGF- β activity was found in both normal and nephritic urine and was expressed in relation to urine creatinine concentration. In the urine of nephritic animals, TGF- β activity was increased from day 2 of disease, peaked on day 7, and returned to normal by day 14. This time course paralleled TGF- β production by isolated nephritic glomeruli. When TGF- β levels for individual animals were compared with the severity of cortical fibrosis, a significant positive correlation was found. The results suggest that urinary TGF- β activity may be a useful predictor of fibrogenesis and progression to end-stage disease.

Puromycin aminonucleoside-induced nephrosis

A single injection of puromycin aminonucleoside into a rat results in an acute, reversible nephrotic syndrome that has been used as a model of minimal-change disease in humans. One week following induction of nephrosis, elevated levels of TGF- β were found in whole kidney tissue that remained increased for 3 weeks [21]. TGF- β is a potent chemoattractant for monocyte-macrophages, and a significant macrophage infiltration was detected in the tubulointerstitium. With activation of TGF- β , there was induction of types I and IV collagen and fibronectin expression in the interstitium along with an increase of the tissue inhibitor of metalloproteinase. These events resulted in transient matrix protein deposition in the interstitium, but when TGF- β expression declined, the histologic appearance

of the tissues returned to normal. The authors suggest that interstitial macrophages may be a major source of TGF- β production in this acute model of nephrosis.

Hyperlipidemia in nephrotic syndrome is alleged to be a progression factor for human glomerular disease. In an interesting report, rats with puromycin aminonucleoside-induced nephrosis were placed on normal or augmented-cholesterol diets [22]. Cholesterol feeding was found to increase glomerular TGF- β expression in the nephrotic rats as well as in normal rats. Increased TGF- β expression also correlated with increased expression of fibronectin, a major component of the mesangial matrix. Again, infiltrating macrophages were thought to be the cells responsible for the TGF- β production. These results suggest a molecular mechanism involving TGF- β by which hyperlipidemia might accelerate the development of glomerulosclerosis.

Diabetic nephropathy

The central pathologic feature of diabetic nephropathy is expansion of the mesangial matrix. In glomeruli of rats made diabetic with streptozotocin, a slow progressive increase in expression of TGF- β mRNA and protein was found [23*]. Matrix proteins known to be induced by TGF- β were deposited within the glomeruli, indicating that TGF- β was biologically active in inducing matrix deposition in the diabetic animals. Administration of insulin reduced TGF- β levels but not to normal levels. These results suggest that hyperglycemia in some way is linked to the elevated TGF- β expression. This is the first report directly implicating a cytokine in the pathogenesis of diabetic nephropathy.

Toxicity studies

Investigators at Genentech Inc. (San Francisco, CA) performed toxicity studies with recombinant TGF- β in rats [24]. They found that glomerulosclerosis developed in 14 days in 30% of rats injected daily with 100 μ g of TGF- β and in 80% injected with 1000 μ g. Liver fibrosis occurred in 50% and 100%, respectively, of the same rats. Thus, the dramatic fibrogenic potential of TGF- β already described and proven in actual models of disease was confirmed by these pharmacologic experiments.

Human glomerulonephritis

Expression of TGF- β in normal and glomerulonephritic kidneys was examined by immunohistochemistry and *in situ* hybridization [25*]. The results are exactly what would be predicted from the animal models previously described. Glomerular staining of TGF- β was strongly

positive in IgA nephropathy and other forms of mesangial proliferative glomerulonephritis. Furthermore, the intensity of TGF- β staining highly correlated with the degree of mesangial matrix expansion. The cells expressing TGF- β were found to be resident glomerular cells rather than infiltrating macrophages.

Human diabetic nephropathy

Kidney tissue from patients with diabetic glomerulosclerosis was examined for TGF- β protein and an isoform of fibronectin known to be induced by TGF- β [23*]. All of the glomeruli from the patients with diabetic glomerulosclerosis were strongly positive for TGF- β and the fibronectin isoform. Control glomeruli from normal individuals and others with minimal-change disease or thin basement membrane disease were negative.

Transforming growth factor- β antagonists as drugs

Antibodies to TGF- β have been successfully employed in the kidney and skin to prevent matrix deposition and scarring [1*]. Recently, the proteoglycan decorin was shown to bind TGF- β and neutralize its action [26]. This finding suggested that decorin might be a natural regulator of TGF- β . Decorin was tested in the model of acute mesangial proliferative glomerulonephritis [27*]. Injections of decorin strongly reduced matrix protein deposition in the nephritic glomeruli, suppressed proteinuria, and ameliorated histologic manifestations of the disease. Suppressing TGF- β did not interfere with normal glomerular healing or have any other deleterious effects. Decorin may be particularly suitable for treatment of kidney disease because of a propensity to accumulate in the kidney following intravenous injection. Also, because decorin is a natural human compound, it offers hope as a treatment for human fibrotic diseases caused by TGF- β .

Conclusions

The findings in cell culture, experimental models, and human disease strongly implicate TGF- β as the major cytokine responsible for extracellular matrix deposition and scarring in glomerular disease. TGF- β antagonists, such as decorin, may eventually be clinically useful in glomerular diseases associated with the overproduction of TGF- β .

Acknowledgments

The original work by the author and the writing of this review were supported by grant DK 43609 from NIDDK-DHHS and a grant from Telios Pharmaceuticals, Inc.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- Of special interest
- Of outstanding interest

1. BORDER WA, RUOSLAHTI E: Transforming Growth Factor- β in Disease: The Dark Side of Tissue Repair. *J Clin Invest* 1992, 90:1-7.
2. BORDER WA, NOBLE NA: Cytokines in Kidney Disease: The Role of Transforming Growth Factor- β . *Am J Kidney Dis* 1993, 22:105-113.
3. SHARMA K, ZIYADEH FN: The Transforming Growth Factor- β System and the Kidney. *Semin Nephrol* 1993, 13:116-128.
4. ABOUD HE: Growth Factors in Glomerulonephritis. *Kidney Int* 1993, 43:252-267.
5. BORDER WA, OKUDA S, LANGUINO LR, RUOSLAHTI E: Transforming Growth Factor- β Regulates Production of Proteoglycans by Mesangial Cells. *Kidney Int* 1990, 37:689-695.
6. NAKAMURA T, MILLER D, RUOSLAHTI E, BORDER WA: Production of Extracellular Matrix by Glomerular Epithelial Cells Is Regulated by Transforming Growth Factor- β 1. *Kidney Int* 1992, 41:1213-1221.
7. HUMES HD, NAKAMURA T, CIESLINSKI DA, MILLER D, EMMONS RV, BORDER WA: Role of Proteoglycans and Cytoskeleton in the Effects of TGF- β 1 on Renal Proximal Tubule Cells. *Kidney Int* 1993, 43:575-584.
8. KANAME S, UCHIDA S, OGATA E, KUROKAWA K: Autocrine Secretion of Transforming Growth Factor- β in Cultured Rat Mesangial Cells. *Kidney Int* 1992, 42:1319-1327.
9. JOHNSON R, IIDA H, YOSHIMURA A, FLOEGE J, BOWEN-POPE DF: Platelet-Derived Growth Factor: A Potentially Important Cytokine in Glomerular Disease. *Kidney Int* 1992, 41:590-594.
10. JAFFER F, SAUNDERS C, SHULTZ P, THROCKMORTON D, WEINSHILL E, ABOUD HE: Regulation of Mesangial Cell Growth by Polypeptide Mitogens: Inhibitory Role of Transforming Growth Factor Beta. *Am J Pathol* 1989, 135:261-269.
11. ABOUD HE: Platelet-Derived Growth Factor and Mesangial Cells. *Kidney Int* 1992, 41:581-583.
12. HABERSTROH U, ZAHNER G, DISSER M, THAISS F, WOLF G, STAHL RA: TGF- β Stimulates Rat Mesangial Cell Proliferation in Culture: Role of PDGF β -Receptor Expression. *Am J Physiol* 1993, 33:F199-F205.
13. OKUDA S, LANGUINO LR, RUOSLAHTI E, BORDER WA: Elevated Expression of Transforming Growth Factor- β and Proteoglycan Production in Experimental Glomerulonephritis: Possible Role in Expansion of the Mesangial Extracellular Matrix. *J Clin Invest* 1990, 86:453-462.
14. MILLER DE, NOBLE NA, YU X, BORDER WA: Molecular and Cellular Biological Techniques in the Study of Glomerular Diseases. *Semin Nephrol* 1992, 12:506-515.

EXHIBIT I

Acute Cyclosporine-Induced Nephrotoxicity in Renal Transplant Recipients: The Role of the Transplanted Kidney¹

Charlotte Wissmann, Felix J. Frey, Paolo Ferrari, and Dominik E. Uehlinger²

C. Wissmann, F.J. Frey, P. Ferrari, D.E. Uehlinger, Division of Nephrology, Department of Medicine, University of Berne, Bern, Switzerland

(J. Am. Soc. Nephrol. 1996; 7:2677-2681)

ABSTRACT

Cyclosporine A causes an acute reduction in GFR. The interindividual variable reduction in GFR is most likely the result of arteriolar vasoconstriction. Vasoconstriction is attributable either to a local effect of cyclosporine on renal blood vessels (intrinsic mechanism) or to a systemic effect of cyclosporine on circulating and/or neuronal factors (extrinsic mechanism). The aim of the investigation presented here was to establish whether intrinsic or extrinsic mechanisms account for the interindividual differences in the susceptibility to acute cyclosporine-induced nephrotoxicity. For that purpose, this study took advantage of the clinical transplant situation in which two (intrinsically identical) kidneys from a cadaveric donor are transplanted into two (extrinsically) different subjects. The preexisting regular daily cyclosporine doses were raised by 25% for 2 wk and by 50% for another 2 wk in 16 patients with stable renal graft function, representing eight pairs of patients, each of whom had received kidneys from the same donor. In these patients, a mean (\pm SD) maximum cyclosporine-induced increase in serum creatinine concentration of $13 \pm 11\%$ ($P < 0.001$) and in serum BUN of $27 \pm 33\%$ ($P < 0.01$), together with a decline in the fractional uric acid excretion of $51 \pm 89\%$ ($P < 0.02$) were observed. The percentage change in serum creatinine concentrations after increased dosing of cyclosporine paralleled within the subjects receiving their kidneys from the same donor, i.e., when one recipient experienced a large percentage of change after increases of cyclosporine dosing, the corresponding recipient of a kidney from the same donor had a change of the same magnitude. Seven of eight pairs showed a consistent response with respect to a clinically significant increase in serum creatinine concentration of

$>15\%$, with a consistent response purely by chance being $<5\%$. Thus, the transplanted kidney itself rather than the recipient determines the susceptibility to acute cyclosporine-induced nephrotoxicity.

Key Words: Cyclosporine, toxicity, renal failure, transplantation, humans

Dose- and time-dependent renal dysfunction (1-3) is the major side effect observed during immunosuppression with cyclosporine. Acute cyclosporine-induced renal dysfunction is nonprogressive, dose-dependent, and reversed by dose reduction or discontinuation (4-6).

The precise pathomechanism underlying cyclosporine's nephrotoxicity remains unclear. Morphologic and functional studies in animals (7-9) and man (10-12) have suggested that increased arteriolar resistance with a predominant afferent arteriolar vasoconstriction is the major mechanism accounting for the acute reduction in GFR when cyclosporine is given. Vasoconstriction might be a result of a local effect of cyclosporine on renal blood vessels or of a systemic effect of cyclosporine on circulating and/or neuronal factors. Thus, the large interindividual differences in the decline of the GFR after cyclosporine dosing (10,11) can be explained either by interindividual differences of the (intrinsic) susceptibility of the kidney itself or by a variable (extrinsic) response of the rest of the body. Donor age (13-15), prolonged warm ischemia time (16,17), and preexisting ischemic injury to cadaver kidneys (18,19) have all been reported as potential risk factors for graft loss. However, nothing is known, to the best of our knowledge, about a potential role of the kidney donor in the individual recipients' susceptibility to cyclosporine-induced nephrotoxicity.

The aim of the investigation presented here was, therefore, to establish whether intrinsic or extrinsic mechanisms account for the interindividual differences in the susceptibility to acute cyclosporine-induced nephrotoxicity. For that reason, we took advantage of the clinical transplant situation in which two (intrinsically identical) kidneys from a cadaveric donor are transplanted into two (extrinsically) different subjects. We hypothesized that a synchronous decline in GFR in pairs of stable kidney transplant recipients by temporarily increasing cyclosporine doses indicates intrinsic organ (or donor) factors, whereas a differential modulation in GFR indicates extrinsic (or recipient) factors.

¹ Received March 28, 1996. Accepted July 3, 1996.

² Correspondence to Dr. D.E. Uehlinger, Division of Nephrology, Department of Medicine, University of Berne, Freiburgstrasse 3, 3010 Bern, Switzerland.

1046-6673/97/072677\$03.00/0

Journal of the American Society of Nephrology

Copyright © 1996 by the American Society of Nephrology

METHODS

Patients

Sixteen patients (nine women, seven men; mean age, 47 years; age range, 28 to 66 yr) with stable functioning renal grafts were studied. These patients represented eight pairs of kidney transplant recipients, each pair with kidneys from the same donor. All recipients were transplanted at least 2 yr before the study. They were on stable long-term immunosuppression with either cyclosporine alone or in combination with prednisone and/or azathioprine (Table 1). All patients had stable renal function and cyclosporine regimen documented by cyclosporine whole blood concentrations, serum creatinine values, and creatinine clearance measurements over the last 2 months before the study.

Patients with diabetes, nonsteroidal anti-inflammatory drug intake and/or uncontrolled hypertension (as defined by a diastolic blood pressure above 105 mm Hg) were excluded. The study was approved by the ethical committee of the University of Berne, and all patients gave their informed consent to undergo the following study protocol.

Study Protocol

The patients remained ambulatory. Cyclosporine was administered orally as a single daily dose, and pre-existing concurrent medications were continued throughout the study. After evaluation of baseline clinical and laboratory parameters (see below), the daily dosage of cyclosporine was raised by 25% for 2 wk. After this period, an additional increase of 25% of the daily dosage of cyclosporine was prescribed for another 2 wk, and thereafter the daily dosage of cyclosporine was reduced back to baseline dosage.

Throughout the study, all patients were seen weekly and the following clinical and laboratory parameters were collected: body weight, blood pressure, heart rate, and, possibly, cyclosporine-induced clinical side effects, such as tremor. Weekly laboratory analyses included cyclosporine whole blood concentrations (trough levels 24 h after the last dose), BUN, serum creatinine, uric acid, potassium, and magnesium levels, as well as total serum bilirubin concentrations. Urine creatinine and uric acid were measured weekly from two 24-h urine collections, and creatinine and uric acid clearances as well as the fractional excretion of uric acid were calculated.

Serum and urine samples were analyzed by standard biochemical assays. Whole blood cyclosporine concentrations were determined by using a specific fluorescence polarization immunoassay (Abbott TDX; Abbott Laboratories, North Chicago, IL) (20).

Criteria for immediate suspension from the study were a

TABLE 1. Patient characteristics^a

Age	47 ± 11
Body Weight (kg)	68 ± 17
Height (cm)	165 ± 9
Blood Pressure (mm Hg)	141/90 ± 18/11
Heart Rate/min	70 ± 10
Immunosuppressive Therapy	
Cyclosporine (mg/day)	273 ± 136
Prednisone (mg/day)	6.2 ± 3.8
Azathioprine (mg/day)	40 ± 54

^a All values given as mean ± SD.

diastolic blood pressure above 105 mm Hg, an increase of serum creatinine levels by more than 50% as compared with baseline values, and/or a rise in serum potassium level above 5.5 mmol/L.

Statistical Analysis

Patients were grouped according to their individual response to the raised cyclosporine doses. An increase of 15% or more of the serum creatinine level during any routine control of a transplanted patient is considered a relevant clinical finding at our division. This same limit was used for the current investigation, and all patients that showed serum creatinine level increase of 15% or more in at least one of the serum creatinine measurements during the study were grouped as "responders." Independent *t* tests were used for comparisons between responders and nonresponders.

RESULTS

The profiled increase of the daily cyclosporine dose by 25% for 2 wk and 50% for another 2 wk resulted in an increase of the daily cyclosporine dose from 271 ± 137 mg/day (mean ± SD) to 341 ± 171 mg/day and to 406 ± 207 mg/day, respectively (Table 2). The corresponding whole blood cyclosporine levels increased from 117 ± 26 to 160 ± 53 and 188 ± 42 ng/mL, respectively. All patients tolerated the increase of the daily cyclosporine dose, and all were able to finish the study protocol.

The mean of the observed individual maximum increases of serum creatinine level during the 4-wk observation period was 13 ± 11% ($P < 0.001$). This increase was accompanied by a 27 ± 33% increase in BUN ($P < 0.01$) and a 51 ± 89% decrease in the fractional excretion of uric acid ($P < 0.05$) (Table 2). Creatinine clearance and serum levels of uric acid, potassium, and magnesium showed no consistent changes.

The patients were grouped according to their individual response to the raised cyclosporine doses. An increase by 15% or more in at least one of the serum creatinine measurements was considered to be of clinical relevance. Nine patients increased their serum creatinine to levels above 15% (responders), whereas in seven patients serum creatinine levels consistently stayed below 15% during the 4 wk of the higher cyclosporine dosage (nonresponders). Responders and nonresponders not only differed with respect to their maximum increase of serum creatinine concentrations (20 ± 3 versus 3 ± 7%, $P < 0.001$) (Table 2) but also with respect to their mean changes in creatinine clearance (−11 ± 6 versus 2 ± 6%, $P < 0.01$) as well as to changes in their maximum BUN concentrations (44 ± 31 versus 4 ± 19%, $P < 0.01$) (Table 2).

A higher percentage increase in cyclosporine blood concentrations was noted for responders at the time of maximum serum creatinine measurements (71 ± 27 versus 40 ± 22%, $P < 0.05$), but the mean increase of cyclosporine blood levels, obtained by considering all of the concentrations after the increase of cyclosporine A dosing, was not significantly different between the two groups (56 ± 20 versus 39 ± 19%). Maximum

TABLE 2. Laboratory values at baseline and at time of maximum increase of serum creatinine concentration

Parameter	All Patients		Responders ^a		Nonresponders	
	Baseline	Maximum ^b	Baseline ^c	Maximum ^b	Baseline ^c	Maximum ^b
Cyclosporine, Whole Blood Concentrations (ng/mL)	117 ± 26	182 ± 48 ^d	108 ± 23	182 ± 46 ^d	129 ± 25	181 ± 53 ^d
Serum Levels						
Creatinine (μmol/L)	126 ± 29	142 ± 32 ^d	124 ± 31	149 ± 35 ^d	129 ± 30	133 ± 28
BUN (mmol/L)	9.9 ± 3.2	12.2 ± 3.9 ^e	9.4 ± 3.3	13.2 ± 4.5 ^d	10.6 ± 3.1	10.8 ± 2.4
Potassium (mmol/L)	4.3 ± 0.7	4.6 ± 0.8	4.2 ± 0.7	4.6 ± 1.0	4.6 ± 0.5	4.6 ± 0.6
Uric Acid (μmol/L)	398 ± 150	436 ± 147	394 ± 161	459 ± 180	404 ± 147	406 ± 95
Magnesium (mmol/L)	0.76 ± 0.09	0.78 ± 0.14	0.78 ± 0.09	0.83 ± 0.13	0.74 ± 0.09	0.71 ± 0.13
Creatinine Clearance (mL/min)	64 ± 31	61 ± 30	73 ± 38	61 ± 24	53 ± 14	62 ± 37
Fractional Uric Acid Excretion (%)	11.2 ± 9.3	7.9 ± 6.3 ^f	12.4 ± 11.4	8.1 ± 8.2	9.5 ± 5.3	7.7 ± 3.1

^a Individual maximum increase of serum creatinine ≥ 15%.

^b At maximum increase of serum creatinine levels.

^c No significant differences between responders and nonresponders were detected for any of the baseline parameters.

^d $P < 0.001$ compared with baseline parameters.

^e $P < 0.01$ compared with baseline parameters.

^f $P < 0.05$ compared with baseline parameters.

serum creatinine concentrations were measured 3.6 ± 1.2 wk after the increase of the cyclosporine dose in responders and 3.0 ± 1.1 wk in nonresponders, respectively.

The 16 patients were grouped into eight pairs, each pair with grafts from the same donor. Pairs with both patients being either responders or nonresponders were defined to exhibit a consistent response to the increase in cyclosporine dosage. Such a consistent response was observed in seven of the eight patient pairs (four responder pairs and three nonresponder pairs) (Figure 1), with the probability of observing seven or more of eight pairs with consistent response purely by chance being less than 5% (9/256).

Patient Pair 5 showed no consistent response to the increase in cyclosporine dosage. Both patients of this pair compared well with respect to all of the investigated baseline parameters. Nevertheless, the one patient that showed no response to the increase in cyclosporine dosage had a stable renal function, but rather large fluctuations in serum creatinine levels of between 155 and 175 μmol/L for months before the study. It is possible that in this patient, the measured baseline serum creatinine of 176 μmol/L was too high, blunting a possible response to the increase in cyclosporine dosage.

No differences between responders or nonresponders were found with respect to baseline laboratory parameters (Table 2), time since transplantation, patient or donor age, patient or donor sex, cold or warm ischemia time, or the current use of the following antihypertensive drugs: calcium channel blockers, angiotensin-converting enzyme inhibitors, betablockers, and/or diuretics (Table 3).

DISCUSSION

Seven of the eight patient pairs with kidneys from the same donor showed consistent responses during the elevated cyclosporine doses with respect to their maximum increase in serum creatinine and BUN

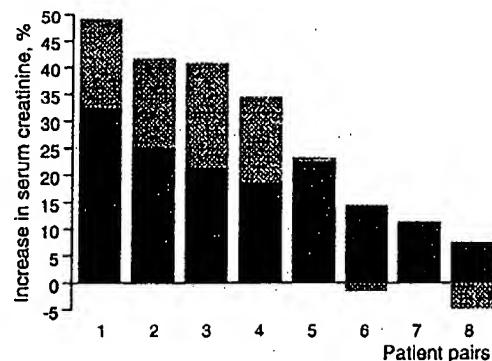


Figure 1. Maximum observed percentage increase in serum creatinine in eight patient pairs, each with grafts from the same donor. Each stacked bar combines the observed changes in serum creatinine level of one patient pair. Visual inspection reveals that the percentage change in serum creatinine levels paralleled within the subjects receiving their kidneys from the same donor, i.e., when one recipient experienced a large percentage change in serum creatinine after increases of cyclosporine A dosing, the corresponding recipient of a kidney from the same donor had a change of the same magnitude. All but one pair (Patient Pair 5) showed consistent creatinine changes in response to the increased cyclosporine doses. One patient of Patient Pair 6 showed a 0% change in serum creatinine value.

TABLE 3. Comparison of parameters in responders and nonresponders^a

	Responders (N = 9)	Non- responders (N = 7)
Patient		
Age ^b	48 ± 12	45 ± 9
Gender (f/m)	4/5	5/2
Donor		
Age ^b	28 ± 6	28 ± 8
Gender (f/m)	2/7	0/7
Time Since Transplantation (yr) ^b	4.3 ± 1.7	5.9 ± 2.2
Cold Ischemia Time (hr) ^b	18 ± 8	16 ± 7
Concomitant Therapy, Number of Patients on Treatment		
Prednisone	8	4
Azathioprine	3	2
Calcium channel blockers	3	4
Beta-blockers	6	5
ACEI ^c	2	3
Diuretics	3	2

^a No significant differences between responders and nonresponders were detected for any of the parameters.

^b Values are given as mean ± SD.

^c ACEI, angiotensin-converting enzyme inhibitors.

levels. The probability of observing such consistent responses in seven or more of eight pairs purely by chance is less than 5% (9 of 256), indicating that the transplanted kidney rather than the recipient accounts for the susceptibility to acute cyclosporine-induced nephrotoxicity. The profiled rise in the cyclosporine dosage during the study was paralleled by corresponding changes in whole blood cyclosporine concentrations. Although kinetic studies suggest that blood concentrations of cyclosporine are of value within certain limits for therapeutic dose finding, measuring cyclosporine blood levels proved useful in monitoring patients compliance, the most important variable of drug efficacy in outpatients (21).

Analysis of all patients as one group revealed a discrete increase of serum creatinine concentrations, whereas creatinine clearance values remained unaltered. These findings might be explained by an augmented tubular secretion of creatinine during the higher cyclosporine doses, with the possible consequence of overestimating the true GFR from creatinine clearance values in cyclosporine-treated patients (22,23). Alternatively, variations in the 24-h urine collections might obscure significant changes in creatinine clearance after increasing cyclosporine A dosage. The higher values of the coefficients of variation for creatinine clearance than for those of plasma concentrations of creatinine (Table 2) are in line with the latter hypothesis.

The observation of a more pronounced increase in BUN levels as compared with serum creatinine concentrations, as well as the decrease in the fractional excretion of uric acid, confirm previous reports that

cyclosporine-induced intrarenal vasoconstriction causes a state of prerenal azotemia. This state results in an increase of the tubular uptake of urea and uric acid, which has been suggested to be a physiological response to the reduction of glomerular filtration pressure and which is completely reversible after drug reduction or discontinuation (24,25).

Several mechanisms have been proposed to participate in the characteristic intrarenal vasoconstriction, including excessive sympathetic nerve stimulation (26), altered eicosanoid metabolism (27-29), either decreased or unchanged activity of the renin-angiotensin system (30,31), alterations in calcium homeostasis by cyclosporine-enhanced transmembrane calcium influx and mobilization from intracellular stores (32,33). In addition, experimental studies suggest that cyclosporine causes exaggerated contractile responses in arteriolar smooth muscles and mesangial cells in the presence of vasoactive substances (34). In renal transplant recipients, calcium channel blockers have been reported to be beneficial in treating cyclosporine-induced impaired renal function and hypertension by counteracting renal vascular constriction or partially inhibiting cyclosporine-induced mesangium-cell contraction (35-37). The concomitant use of vasoactive drugs might therefore be an important determinant for the susceptibility to cyclosporine-induced renal dysfunction. In the investigation presented here, no relationship between cyclosporine-induced renal dysfunction and the use of calcium channel blockers, betablockers, angiotensin-converting enzyme inhibitors, and/or diuretics was detected.

The differences detected between responders and nonresponders could not be related to any of the studied recipient- or donor-specific covariates. However, the observed differences were rather discrete and several parameters known to be related with acute cyclosporine-induced renal dysfunction, such as serum potassium (38,39) and bilirubin levels (40), did not significantly increase in the responder group during the higher cyclosporine doses. More pronounced differences might have been observed with higher cyclosporine doses and/or a prolonged observation period. For ethical reasons, we did not take the risk to increase steady-state cyclosporine doses by more than 50% in these patients with stable renal function or to increase the number of subjects to be investigated. It should be noted that short-term cyclosporine administration often showed its nephrotoxic effects immediately after exposure (41-43).

In conclusion, this investigation demonstrates that the transplanted kidney itself and not the recipient determines the susceptibility to acute cyclosporine-induced toxicity.

ACKNOWLEDGMENTS

We are grateful to all of our patients who participated in this study. It is anything but an obvious choice for a patient with a renal graft to accept the possibility of a transient decrease in renal function for the pure sake of science. This study was supported by Grant 32-3641.92 of The Swiss National Science Foundation.

- RE
- 1.
- 2.
- 3.
- 4.
- 5.
- 6.
- 7.
- 8.
- 9.
- 10.
- 11.
- 12.
- 13.
- 14.
- 15.
- 16.
- 17.
- 18.
- 19.
- 20.
- 21.
- 22.

REFERENCES

- Kahan BD, Oates JA, Wood AJJ: Cyclosporine. *N Engl J Med* 1989;321:1725-1738.
- Remuzzi G, Bertani T: Renal vascular and thrombotic effects of cyclosporine. *Am J Kidney Dis* 1989;13:261-272.
- Kopp JB, Klotmann PE: Cellular and molecular mechanisms of cyclosporine nephrotoxicity. *J Am Soc Nephrol* 1990;1:162-179.
- Chapman JR, Griffiths D, Harding NG, Morris PJ: Reversibility of cyclosporine nephrotoxicity after three months' treatment. *Lancet* 1985;1:128-130.
- Flechner SM, Van Buren C, Kerman RH, Kahan BD: The nephrotoxicity of cyclosporine in renal transplant recipients. *Transplant Proc* 1983;15(Suppl 1):2689-2694.
- Feutren G, Abeywickrama K, Friend D, Von Graffenreid B: Renal function and blood pressure in psoriatic patients treated with cyclosporine A. *Br J Dermatol* 1990;122(Suppl 36):57-69.
- Barros EJG, Boim MA, Ajzen H, Ramos OL, Schor N: Glomerular hemodynamics and hormonal participation on cyclosporine nephrotoxicity. *Kidney Int* 1987;32:19-25.
- Murray BM, Paller MS, Ferris TF: Effect of cyclosporine administration on renal hemodynamics in conscious rats. *Kidney Int* 1985;28:767-774.
- Bennett WM, Houghton DC, Buss WC: Cyclosporine-induced renal dysfunction: Correlations between cellular events and whole kidney function. *J Am Soc Nephrol* 1991;1:1212-1219.
- McNally PG, Feehally J: Pathophysiology of cyclosporin A nephrotoxicity: Experimental and clinical observations. *Nephrol Dial Transplant* 1992;7:791-804.
- Myers BD: Cyclosporine nephrotoxicity. *Kidney Int* 1986;30:964-974.
- Curtis JJ, Dubovsky E, Whelchel JD, Luke RG, Diethelm AG, Jones P: Cyclosporin in therapeutic doses increases renal allograft vascular resistance. *Lancet* 1986;2:477-479.
- Higgins RM, Sheriff R, Bittar AA, et al.: The quality of function of renal allografts is associated with donor age. *Transplant Int* 1995;8:221-225.
- Rao KV, Kasiske BL, Odlund MD, Ney AL, Andersen RC: Influence of cadaver donor age on posttransplant renal function and graft outcome. *Transplantation* 1990;49:91-95.
- Lucas BA, Vaughn WK, Spees EK, Sanfilippo F: Identification of donor factors predisposing to high discard rates of cadaver kidneys and increased graft loss within one year posttransplantation. *Transplantation* 1987;43:253-257.
- The Canadian Multicenter Transplant Study Group: A randomized clinical trial of cyclosporine in cadaver renal transplantation. *Lancet* 1986;314:1219-1225.
- Pappalettera M, Pizzi C, Cardillo M, et al.: Factors influencing cadaver kidney graft survival in two cyclosporine periods. *Transplant Proc* 1994;26:2533-2534.
- Hall BM, Tiller DJ, Duggin GG, et al.: Post-transplant acute renal failure in cadaver renal recipients treated with cyclosporine. *Kidney Int* 1985;28:178-186.
- Seron D, Carrera M, Grino JM, Castelao AM, Lopez-Coste MA, Riera L: Relationship between donor renal interstitial surface and posttransplant function. *Nephrol Dial Transplant* 1993;8:539-543.
- Beutler D, Molteni S, Zeugn T, Thormann W: Evaluation of instrumental, nonisotopic immunoassays (fluorescence polarization immunoassay and enzyme-multiplied immunoassay technique) for cyclosporine monitoring in whole blood after kidney and liver transplantation. *Ther Drug Monit* 1992;14:424-432.
- Frey FJ: Pharmacokinetic determinants of cyclosporine and prednisone in renal transplant patients. *Kidney Int* 1991;39:1034-1050.
- Tomlanovich S, Golbetz H, Perlroth M, Stinson E, Myers BD: Limitations of creatinine in quantifying the severity of cyclosporine-induced chronic nephropathy. *Am J Kidney Dis* 1986;8:332-337.
- Shemesh O, Golbetz H, Kriss JP, Myers BD: Limitations of creatinine as a filtration marker in glomerulopathic patients. *Kidney Int* 1985;28:830-838.
- Gupta AK, Rocher LL, Schmaltz SP, et al.: Short-term changes in renal function, blood pressure, and electrolyte levels in patients receiving cyclosporine for dermatologic disorders. *Arch Intern Med* 1991;151:356-362.
- Laskow DA, Curtis JJ, Luke RG, et al.: Cyclosporine-induced changes in glomerular filtration rate and urea excretion. *Am J Med* 1990;88:497-502.
- Scherrer U, Vlissing SF, Morgan BJ, et al.: Cyclosporine-induced sympathetic activation and hypertension after heart transplantation. *N Engl J Med* 1990;323:693-699.
- Smith SR, Creech EA, Schaffer AV, et al.: Effects of thromboxane synthase inhibition with CGS 13080 in human cyclosporine nephrotoxicity. *Kidney Int* 1992;41:199-205.
- Pouteil-Noble C, Chapuis F, Berra N, et al.: Misoprostol in renal transplant recipients: A prospective, randomized, controlled study on the prevention of acute rejection episodes and cyclosporin A nephrotoxicity. *Nephrol Dial Transplant* 1994;9:552-555.
- Van der Heide JJJ, Bilo HJG, Donker JM, Wilmsink JM, Tegzess AM: Effect of dietary fish oil on renal function and rejection in cyclosporine-treated recipients of renal transplants. *N Engl J Med* 1993;329:769-773.
- Stanek B, Kovarik J, Rasoul-Rockenschaub S, Silberbauer K: Renin-angiotensin-aldosterone system and vasoressin in cyclosporine-treated renal allograft recipients. *Clin Nephrol* 1987;28:186-189.
- Bantle JP, Boudreau RJ, Ferris TF: Suppression of plasma renin activity by cyclosporine. *Am J Med* 1987;83:59-64.
- Zidek W, Neumann KH: Calcium release in permeabilized neutrophils induced by cyclosporine. *Nephron* 1990;56:30-34.
- Pfeilschifter J, Rüegg UT: Cyclosporine A augments angiotensin II-stimulated rise in intracellular free calcium in vascular smooth muscle cells. *Biochem J* 1987;248:883-887.
- Meyer-Lehnert H, Schrier RW: Cyclosporine A enhances vasoressin-induced calcium mobilization and contraction in mesangial cells. *Kidney Int* 1988;34:89-97.
- Dawidson I, Rooth P: Improvement of cadaver renal transplantation outcomes with verapamil: A review. *Am J Med* 1991;90:37S-41S.
- McNally PG, Walls J, Feehally J: The effect of nifedipine on renal function in normotensive cyclosporine-A-treated renal allograft recipients. *Nephrol Dial Transplant* 1990;5:962-968.
- Wagner K, Albrecht S, Neumayer H-H: Prevention of posttransplant acute tubular necrosis by the calcium antagonist diltiazem: A prospective randomized study. *Am J Nephrol* 1987;7:287-291.
- Adu D, Michael J, Turney J, McMaster P: Hyperkalemia in cyclosporin-treated renal allograft recipients. *Lancet* 1983;2:370-371.
- Mason J: Renal side-effects of cyclosporin A. *Br J Dermatol* 1990;122(Suppl 36):71-77.
- Bluhm RE, Rodgers WH, Black DL, Wilkinson GR, Branch R: Cholestasis in transplant patients—what is the role of cyclosporin? *Aliment-Pharmacol-Ther* 1992;6:207-219.
- Deray G, Benhmidha M, Le Hoang P, et al.: Renal function and blood pressure in patients receiving long-term, low-dose cyclosporine therapy for idiopathic autoimmune uveitis. *Ann Intern Med* 1992;117:578-583.
- Ruggenenti P, Perico N, Mosconi L, et al.: Calcium channel blockers protect transplant patients from cyclosporine-induced daily renal hypoperfusion. *Kidney Int* 1993;43:706-711.
- Fulano G, Sepe V, Cianfrone P, et al.: Acute effects of low-dose cyclosporine on renal function in normal subjects. *Transplantation* 1991;51:734-736.

EXHIBIT K

Nonsteroidal Anti-Inflammatory Drugs: Effects on Kidney Function

Andrew Whelton, MD, FACP, FCP, and Cindy W. Hamilton, PharmD

Nonsteroidal anti-inflammatory drugs (NSAIDs) are capable of inducing a variety of renal function abnormalities, particularly in high-risk patients with decreased renal blood perfusion who depend on prostaglandin synthesis to maintain normal renal function. Fluid retention is the most common NSAID-related renal complication, occurring to some degree in virtually all exposed individuals; however, clinically detectable edema occurs in less than 5% of patients and is readily reversible on discontinuation of the NSAID. Other electrolyte complications, notably hyperkalemia, are seen infrequently and occur in specific at-risk patients. The next most worrisome complication is acute deterioration of renal function, which occurs in high-risk patients and is also reversible. Nephrotic syndrome with interstitial nephritis is a rare problem of NSAID use and is reversible. Papillary necrosis is the only permanent complication of NSAIDs and is very rare. Altogether, these renal function abnormalities, with the exception of mild fluid retention, are clinically detectable in approximately 1% of exposed patients. Given the number of patients who take NSAIDs on a prescription or over-the-counter basis, the absolute number of at-risk patients is relatively large. Consequently, an appreciation for the risk factors and pathophysiology of NSAID-induced renal function abnormalities is required for optimal use of these drugs.

Approximately 1-5% of persons who are exposed to a nonsteroidal anti-inflammatory drug (NSAID) will manifest one of a variety of renal function abnormalities. Although this percentage appears relatively low, the number of at-risk individuals is enormous because of the current use profile of NSAIDs, either as prescription or over-the-counter drugs. One in seven Americans is likely to be treated with an NSAID for a chronic rheumatologic disorder. If patients who take NSAIDs for acute problems are considered, the exposure rate will be even higher. Thus, of the 50 million Americans expected to use NSAIDs intermittently or routinely this year, at least 500,000 are likely to develop some degree of renal functional abnormality.

In descending order of frequency, the primary NSAID-related renal abnormalities are 1) fluid and electrolyte disturbances, 2) acute deterioration of renal function, 3) nephrotic syndrome with intersti-

tial nephritis, and 4) papillary necrosis (Table I). Sodium chloride and water retention, the most commonly encountered renal effect of NSAID use, occurs to some degree in virtually all exposed persons but results in clinically detectable edema in less than 5% of patients. This rate is probably higher in selected at-risk patients. NSAID-induced fluid retention is typically benign, reversible on discontinuation of the NSAID, and easily managed in patients who require treatment. Other electrolyte abnormalities are also induced by NSAIDs, the most important of which is potassium retention and hyperkalemia. A high-risk group can also be identified for this electrolyte abnormality.

From the clinical point of view, the most worrisome renal side effect of NSAIDs is hemodynamically mediated acute renal failure, which occurs in individuals with pre-existing reduced renal blood perfusion. Ordinarily, the kidneys of such at-risk patients produce vasodilatory prostaglandins to maintain renal perfusion and function. The inhibitory effects of NSAIDs on renal prostaglandin production lead to acute, reversible renal failure in these patients. Acute deterioration of renal function occurs in 0.5 to 1% of patients who take NSAIDs on a chronic basis.

From the Department of Medicine (Dr. Whelton), Johns Hopkins University School of Medicine, Baltimore, Maryland, and Virginia Beach (Dr. Hamilton), Virginia. Address for reprints: Andrew Whelton, MD, The Johns Hopkins Hospital, 1830 East Monument Street, Rm 815, Baltimore, MD 21205.

TABLE I
Documented Renal Effects of Nonsteroidal Anti-Inflammatory Drugs

Drug Class	Generic Name	Brand Name/ Manufacturer	Renal Effects*				
			Edema	†K	ARF	NS	PN
Salicylates	Aspirin	(various)	Cl		Cl		Cl
	Diflunisal	Dolobid/Merck	Cl		Cl	Cl	An
Propionic acids	Ibuprofen	Motrin/Upjohn	Cl	Cl	Cl	Cl	Cl
	Naproxen	Naprosyn/Syntex	Cl		Cl	Cl	An
Indolacetic acids	Fenoprofen calcium	Nalfon/Lilly	Cl		Cl	Cl	Cl
	Ketoprofen	Orudis/Wyeth-Ayerst	Cl		Cl	Cl	
Anthranilic acids	Flurbiprofen	Ansaid/Upjohn	Cl	Cl	Cl	Cl†	An
	Indomethacin	Indocin/Merck	Cl	Cl	Cl	Cl	Cl‡
Pyrazolones	Sulindac	Clinoril/Merck	Cl	Cl	Cl	Cl	An
	Tolmetin	Tolectin/McNeil	Cl		Cl	Cl	An
Oxicams	Diclofenac	Voltaren/Ciba-Geigy	Cl		Cl	Cl	Cl
	Meclofenamate sodium	Meclofenamate/Parke-Davis	Cl		Cl	Cl	An
Phenylbutazone	Mefenamic acid	Ponstel/Parke-Davis	Cl		Cl	Cl	Cl
	Piroxicam	Butazolidin/Ciba-Geigy	Cl		Cl	Cl	Cl
Oxicams	Feldene/Pfizer	Feldene/Pfizer	Cl	Cl	Cl	Cl	Cl

* ARF = acute renal failure; NS = interstitial nephritis and nephrotic syndrome; PN = papillary necrosis; †K = hyperkalemia; Cl = reported in clinical studies; An = described in studies in animals (but not in humans).

† Causes interstitial nephritis without nephrotic syndrome.

‡ Reported in combination with phenylbutazone.
(Adapted from Clive and Stoff,¹ with permission.)

The nephrotic syndrome, with associated interstitial nephritis, is seen on rare occasions. Once again, it is reversible on discontinuation of the NSAID in question.

According to the respective manufacturers' prescribing information, chronic administration of nearly all NSAIDs produces papillary necrosis in laboratory animals; and a few clinical case reports of papillary necrosis can be found in the recent medical literature. Within the framework of our present understanding of NSAID effects on the kidney, this appears to be the only irreversible form of renal toxicity.

Many of the renal abnormalities that are encountered as a result of NSAID use can be attributed to the action of these drugs on prostaglandins. Hence, a brief overview of the interactions between prostaglandins and renal function will be presented, followed by an analysis of the pathophysiology, clinical manifestations, patient risk factors, and preventive approaches to NSAID-induced renal syndromes.

THE PROSTAGLANDIN PATHWAY

Prostaglandins are ubiquitous substances that influence renal function along with a variety of other body systems.^{1,2} Conceptually, they may be considered local hormones or "autocoids" because they act in a paracrine or autocrine fashion. Biologic activity is limited to the site of action by the short half-life of

prostaglandins in circulation. In addition, prostaglandins are not stored in tissue, but are synthesized on demand.

Prostaglandins are derived from phospholipids by a common pathway (Figure 1). Phospholipids, of course, are widely distributed in cell membranes throughout the body. The most important precursor for prostaglandins is arachidonic acid. Cyclooxygenase is the catalyst for oxygenation of arachidonic acid, which is the step that is inhibited by NSAIDs. The interaction between aspirin and cyclooxygenase (acetylation) is irreversible, whereas that with other NSAIDs is reversible.

Arachidonic acid can also be metabolized to other mediators, depending on the cell type. For example, lipoxygenase catalyzes the production of leukotrienes, and mixed-function oxygenases catalyze the production of epoxyeicosatrienoic acids. Collectively, these oxygenated metabolites of arachidonic acid are known as eicosanoids because of their origin from a 20-carbon (eicoso-) polyunsaturated acid.³

Continuing along the common pathway (Figure 1), oxygenation of arachidonic acid results in production of prostaglandin G₂, which is converted to prostaglandin H₂ by hydroperoxidase and loss of a free radical. At this point, metabolism becomes highly specific for individual cell types, although many, if not all, of the metabolites are produced in the kidney. Prostaglandin E₂ is a vasodilator, which, in the kidney, promotes diuresis and natriuresis. Prostaglan-

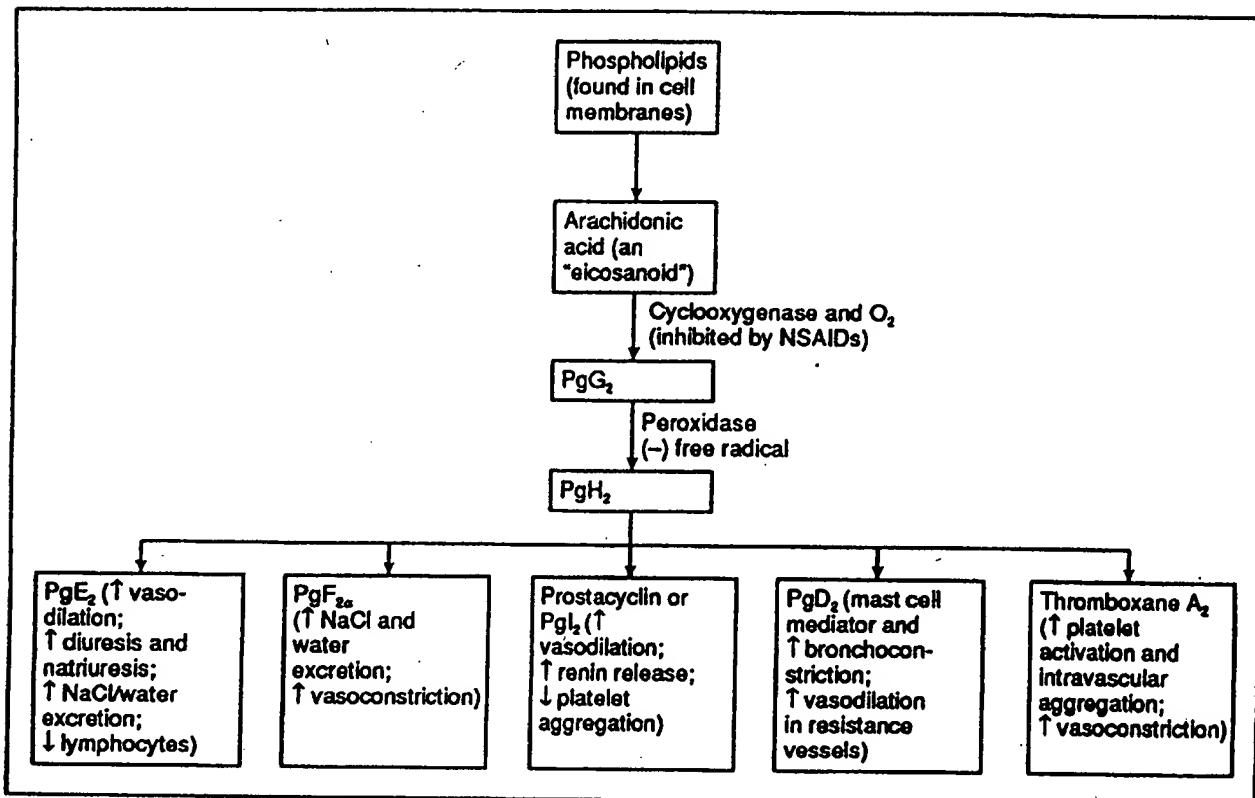


Figure 1. Prostaglandin pathway (and prostanoid functions). Pg = prostaglandin; ↑ = stimulate or increase; ↓ = inhibit or decrease.^{1,2}

din E₂ also inhibits lymphocytes and other cells that are involved in inflammation and allergic responses, which, as will be discussed later, may play a role in some NSAID-induced renal syndromes. Prostaglandin F_{2α} enhances excretion of sodium chloride and water. Prostacyclin, also known as prostaglandin I₂, has a wide variety of actions including vasodilation, renin release, and inhibition of platelet aggregation. Prostaglandin D₂ is a vasodilator of peripheral resistance vessels but is better known for its association with mast cell activation and bronchoconstriction. Thromboxane A₂ is the principal metabolite of prostaglandin H₂ in platelets and can act as a major vasoconstrictor within the kidney. These pharmacologically active metabolites of prostaglandin H₂ are collectively known as prostanoids.³

PROSTAGLANDIN EFFECTS ON RENAL FUNCTION

Given the diversity of cell populations within the kidney and their various functions, the complexity of the interactions between prostaglandins and renal function is not unexpected. Prostaglandins are involved in renin release, local vascular tone, regional

circulation, sodium and water homeostasis, and potassium balance (Table II). The following sections describe these diverse effects. Detailed overviews of these interactions can be found in excellent reviews by Patrono and Dunn² and Oates and colleagues.³

An important caveat in the following sections is that prostaglandins are not primary mediators of basal renal function in normal individuals. Prostaglandins typically operate in conjunction with a variety of other mediators, which, even in the absence of prostaglandins, can preserve homeostasis. Prostaglandin production is increased as needed in response to stress (e.g., decreased renal blood flow or blood volume). Thus, inhibition of prostaglandin function by NSAIDs is more likely to cause complications in at-risk patients with decreased renal blood perfusion than in the otherwise normal subject whose prostaglandins are merely one of many factors contributing to homeostasis.

Renin Release

Prostaglandins stimulate renin release, which plays an important role in the regulation of arterial blood

TABLE II

Principal Renal Sites of Prostaglandin Synthesis and Major Actions

Site	Eicosanoid	Action
Vasculature	Prostaglandins I_2 and D_2	Vasodilation
Glomerulus	Prostaglandins I_2 and E_2	Maintain GFR
Collecting tubule	Thromboxane A_2 Prostaglandins E_2 and $F_{2\alpha}$	Reduce GFR Enhance excretion of sodium chloride and water
Medullary interstitial cells	Prostaglandin E_2	Vasodilation and natriuresis-diuresis

(Adapted from Patrono and Dunn², with permission.)

pressure, blood volume, and electrolyte balance. Prostaglandins can act independently or synergistically with the β -adrenergic system.⁴ Although the exact prostanoid mediator is not yet known, it is likely that prostacyclin is synthesized in response to a change in arteriole pressure or chloride reabsorption in the macula densa of the nephron.³

Local Vascular Tone

Prostanoids are one of several local mediators that govern vascular tone through their actions on norepinephrine release at peripheral nerve endings. Prostaglandins E_2 and D_2 , and, to a lesser extent, prostacyclin promote vasodilation by inhibiting norepinephrine release. Prostaglandin E_2 also antagonizes the effects of angiotensin II, a powerful vasoconstrictor, on the neuroeffector junction. Conversely, prostaglandin $F_{2\alpha}$ and thromboxane A_2 are vasoconstrictors.³

Regional Circulation

Prostanoids contribute to regional circulation via their influence on local vascular tone. Under normal conditions, prostanoids do not regulate renal perfusion per se. However, certain conditions such as decreased renal blood flow are associated with the production of vasodilatory prostaglandins. Prostaglandin E_2 , prostacyclin, and prostaglandin D_2 , shift regional blood flow from cortical to juxtamedullary nephrons.³

Sodium and Water Homeostasis

All prostanoids are capable of acting in the renal cortex to regulate sodium and water homeostasis; however, prostanoids are only one of many factors that share this function.³ Prostaglandins E_2 and D_2 , prostacyclin, and, to a lesser extent, prostaglandin $F_{2\alpha}$, increase the rate of salt and water excretion. Prostaglandin E_2 inhibits sodium chloride transport in the thick ascending limb of the loop of Henle and the collecting duct.^{3,6} In addition, prostaglandins antagonize the effects of antidiuretic hormone.^{7,8}

Prostanoids do not have a direct effect on glomerular filtration rate; however, vasodilation associated with prostaglandin E_2 , prostacyclin, and prostaglandin D_2 , increases renal blood flow, and, as previously mentioned, shunts blood flow from the cortical to juxtamedullary nephrons. The net result is enhanced diuresis and natriuresis due to reduced medullary hypertonicity and increased interstitial pressure.³

Potassium Balance

Prostanoids indirectly lower potassium by their effects on glomerular filtration and renin.³ As previously mentioned, vasodilatory prostaglandins increase renal blood flow. This may enhance the direct intratubular delivery of potassium into the distal nephron for excretion. Alternatively, this may serve to quantitatively increase sodium delivery into the distal nephron with resultant reabsorption of sodium in exchange for potassium, which is then excreted in the urine. Secondly, prostacyclin is believed to promote renin release. Activation of the renin-angiotensin pathway ultimately causes aldosterone to stimulate potassium excretion in the distal convoluted tubule and collecting duct. However, potassium balance is also regulated by a number of other factors such as insulin and the β -adrenergic system.

FLUID AND ELECTROLYTE DISTURBANCES

Sodium and Water Retention

The most common and universal renal complications of NSAIDs are sodium retention and edema. According to prescribing information accompanying nearly all NSAIDs, edema occurs in at least 3% of patients. The incidence is probably higher in patients who take therapeutic doses over prolonged periods. The onset of fluid retention usually occurs early in the course of therapy and can be dramatic as

illustrated by the 15-kg weight gain in a 70-year-old man who took ibuprofen for only 17 days.⁹

Occasionally, the patient may retain water in excess of sodium. Severe, reversible hyponatremia (118 $\mu\text{mol Na}^+/\text{L}$) occurred in a patient who took ibuprofen for only 3 days. This patient had underlying renal impairment (CrCl 12 mL/min).¹⁰

The multiple mechanisms by which NSAIDs interfere with water and sodium metabolism may explain the frequency of this complication. As previously mentioned, NSAIDs have the potential to disrupt diuresis and natriuresis by interfering with prostaglandin-mediated sodium chloride transport, antidiuretic hormone, and distribution of blood flow from cortical to juxtamedullary nephrons.^{1,3} The hypothesis for the pathogenesis of the nephrotic syndrome is also operative in this situation. By shunting arachidonic acid metabolism from prostaglandins to lipoxygenase products, NSAIDs may favor production of eicosanoid derivatives that increase capillary permeability.¹

Hyperkalemia

Hyperkalemia is an unusual complication of NSAIDs, presumably because of the multiplicity of factors that are capable of maintaining potassium balance, even in the absence of prostaglandins. Hyperkalemia is more likely to occur in patients with pre-existing renal impairment,^{11,12} cardiac failure,¹³ diabetes,¹² or multiple myeloma¹⁴ or in patients who receive potassium supplementation,¹⁵ potassium-sparing diuretics,¹⁶ or angiotensin-converting enzyme (ACE) inhibitors. Indomethacin appears to be the major NSAID associated with this complication and has produced hyperkalemia in patients without apparent risk factors.¹⁷ Thus, indomethacin may have a direct effect on the cellular uptake of potassium,¹⁸ in addition to the known effects of NSAIDs on potassium delivery to the distal tubule as well as on the renin-angiotensin and aldosterone pathways.

NSAID-induced hyperkalemia often occurs in the setting of NSAID-induced acute renal deterioration or worsening of underlying renal impairment. However, the severity of hyperkalemia can be disproportionate to that of renal impairment. For example, Tan and colleagues reported a patient who was treated with indomethacin and had a serum potassium of 6.2 mEq/L in spite of only mildly abnormal renal function.¹⁹ In this patient, plasma renin and aldosterone levels were suppressed and did not respond to furosemide or postural changes. Urinary prostaglandin E_2 was also suppressed. Discontinuation of indomethacin resulted in normalization of po-

tassium, prostaglandin E_2 , and a rebound of renin and aldosterone.

ACUTE DETERIORATION OF RENAL FUNCTION

Role of Prostanoids in Maintaining Renal Blood Flow

Although NSAIDs do not impair glomerular filtration in normal individuals,^{20,21} acute renal decompensation may occur in at-risk patients with various extra-renal or renal disease processes that lead to decreased renal perfusion (Table III). Renal prostaglandins play an important role in the maintenance of homeostasis in these patients, so drug-induced disruption of counter-regulatory mechanisms can produce clinically important and even severe renal functional deterioration.^{2,3}

Acute renal deterioration in this setting can be attributed to the interruption of the delicate balance between hormonally mediated pressor mechanisms and prostaglandin-related vasodilatory effects (Figure 2). In at-risk patients, volume contraction triggers pressor responses via adrenergic and renin-angiotensin pathways. Ordinarily, vasodilatory renal prostaglandins counterbalance the vasoconstrictive effects of norepinephrine and angiotensin II. The addition of NSAIDs increases the risk of azotemia and possibly ischemic damage to the kidney by removing the protective effects of vasodilatory prostaglandins and allowing unopposed vasoconstriction.

Clinical Features of Acute Renal Failure

Initially, this NSAID-induced renal syndrome is of moderate severity and is characterized by increasing BUN, creatinine, potassium, and weight with decreasing urine output. NSAID-induced acute renal failure is usually reversible over 2 to 7 days after discontinuation of therapy; however, morbid consequences can occur if the diagnosis is not recognized early. Continued NSAID therapy in the setting of de-

TABLE III

At-Risk Patients for NSAID-Induced Acute Renal Failure

- Severe heart disease (congestive heart failure)
- Severe liver disease (cirrhosis)
- Nephrotic syndrome (chronic renal disease)
- Elderly population
- Dehydration (protracted)

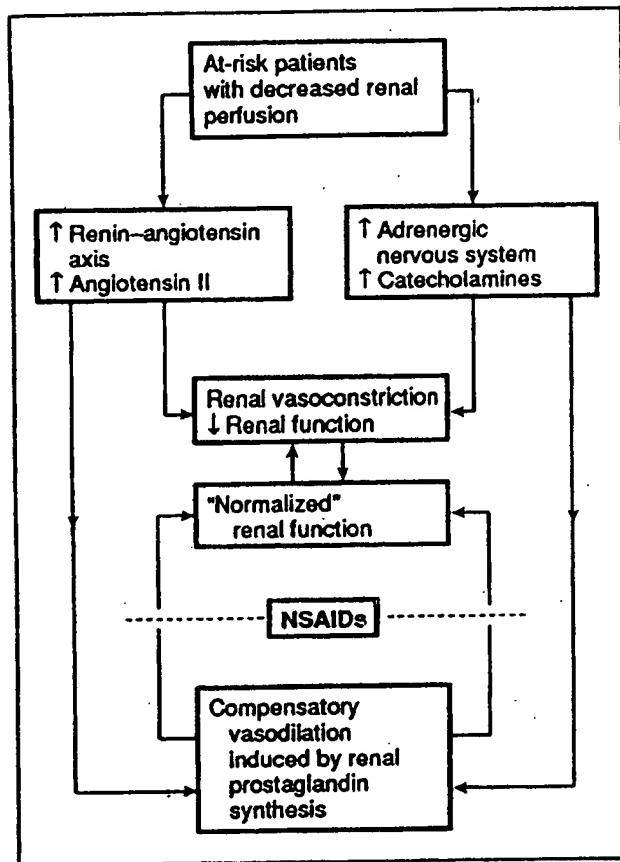


Figure 2. Mechanism by which NSAIDs disrupt the compensatory vasodilation response of renal prostaglandins to vasoconstrictor hormones in patients with prerenal conditions. A solid line (—) indicates stimulation; a dashed line (---) indicates inhibition.

teriorating renal function may progress rapidly to the point wherein dialysis support is required.²² Despite this profound level of renal functional impairment, the kidney will nonetheless recover several days to weeks after discontinuation of the NSAID. Development of this type of "total" renal failure, which is often inappropriately designated as "acute tubular necrosis," represents the extreme end of the spectrum of hemodynamic insult rather than a separate clinical entity.

Risk Factors for Acute Renal Failure

The risk of acute renal deterioration is highest in patients with liver disease, pre-existing renal impairment, cardiac failure, protracted volume contraction due to diuretic therapy or intercurrent disease, or old age. NSAID-induced renal decompensation has been well documented in patients with cirrhosis, par-

ticularly when ascites is present.³ Urinary excretion of prostaglandin E₂, prostacyclin metabolites, and thromboxane A₂ is increased in these patients.^{23,24} An analogous situation exists in patients with underlying congestive heart failure,²⁵ nephrotic syndrome,^{26,27} or lupus nephritis.^{28,29}

Patients with chronic renal impairment are at increased risk of NSAID-induced renal failure because of inadequate renal prostaglandin production. We documented NSAID-induced acute renal failure in patients with asymptomatic mild, but chronic, renal failure (serum creatinine between 1.5 and 3.0 mg/dL).³⁰ Baseline excretion of urinary prostaglandin E₂ and 6-keto-prostaglandin F_{1α} was quantitatively lower in the individuals who developed NSAID-induced renal decompensation than in those who did not, and ibuprofen proved to be more problematic than either piroxicam or sulindac. On initiation of ibuprofen, urinary prostaglandin excretion fell in all patients, but trough concentrations were quantitatively lower in the subset of patients who experienced acute renal failure.

Volume contraction due to diuretic therapy or an intercurrent disease that results in dehydration represents another important risk factor for the development of NSAID-induced acute deterioration of renal function.^{22,31,32} Elderly patients are also at increased risk. We estimate that age of 80 years or greater is an independent risk factor because the physiology of ageing within the kidney results in 50% loss of function in 50% of the population at age 80, primarily as a result of the progression of arteriolonephrosclerosis.

Pharmacodynamics of Acute Renal Failure

NSAID-induced acute renal decompensation is a pharmacologically predictable phenomenon that occurs in a dose-related fashion. In our triple-crossover study of 12 women with mild renal failure, ibuprofen (800 mg three times daily) was discontinued on day 8 because of worsening renal function (≥ 1.5 mg/dL increase in serum creatinine) or hyperkalemia (potassium ≥ 6 mEq/mL) in 3 patients. When these patients were rechallenged at a 50% lower dose of ibuprofen, two patients again had evidence of acute renal deterioration.³⁰

Another important finding in our study was the time of onset of acute renal decompensation.³⁰ Ibuprofen-induced renal failure occurred rapidly (within days), but piroxicam and sulindac did not cause renal deterioration during the 11-day treatment period. A pharmacokinetic analysis in these patients provides insight. Ibuprofen, which has a short elimination half-life, reached maximum serum

concentrations quickly. In contrast, piroxicam and sulindac have longer half-lives and continued to accumulate throughout the treatment period. These findings are consistent with basic pharmacologic principles and suggest that NSAIDs having short elimination half-lives will reach steady state and exert maximum pharmacologic effects before NSAIDs having longer half-lives.

"Renal Sparing" NSAIDs — ?

Although all NSAIDs have the potential to induce acute renal impairment, some quantitative differences may exist. Sulindac has been hypothesized to be renal sparing, possibly because of its unusual metabolic pathway.³³ The parent compound, sulindac sulfoxide, is an inactive prodrug that undergoes hepatic metabolism to sulindac sulfide, which is the metabolite that exerts anti-inflammatory activity. Sulindac sulfoxide is also metabolized to a much lesser extent to an inactive metabolite, sulindac sulfone. It has been hypothesized that, within the kidney, sulindac sulfide is reversibly oxidized to the inactive parent compound, sulindac sulfoxide, such that renal prostaglandin production would not be influenced.

In clinical studies, urinary prostaglandin levels and renal effects were unchanged in patients with normal renal function^{34,35} and states of proteinuria.³⁶ However, the duration of sulindac in these studies may have been insufficient to appreciate the full pharmacologic effect of sulindac. NSAID-induced changes may not have been detectable because of the presence of only very mild renal impairment or absence of renal failure altogether in these studies. Longer courses of sulindac in patients with slightly more severe renal impairment have been associated with statistically significant reductions in urinary prostaglandins³⁰ and glomerular filtration rate.³⁷

The ability of sulindac to inhibit prostaglandin synthesis and impair renal function has been confirmed in a different high-risk group, namely patients with hepatic cirrhosis and ascites.³⁸ We have also identified the development of profound acute renal failure in high-risk patients who received sulindac for several days to weeks. Collectively, these clinical experiences indicate the need for cautious and timely monitoring of high-risk patients who receive NSAIDs.

NEPHROTIC SYNDROME WITH INTERSTITIAL NEPHRITIS

NSAIDs also cause another type of renal dysfunction that is associated with various levels of functional

impairment and characterized by the development of the nephrotic syndrome with interstitial nephritis.^{1,22,39,40} The clinical features, absence of risk factors, and pathophysiology distinguish this from other NSAID-induced renal syndromes and from classic drug-induced allergic interstitial nephritis.

The features of this NSAID-induced renal syndrome are variable. The patient may experience edema, oliguria, and/or foamy urine.⁴¹ Systemic signs of allergic interstitial nephritis such as fever, drug rash, peripheral eosinophilia, and eosinophiluria are generally absent.^{1,22,40,41} The urine sediment contains microscopic hematuria and pyuria.^{1,41} Proteinuria typically is in the nephrotic range.^{1,39} We have noted that renal functional deterioration can range from minimal to severe.

Characteristically, this form of nephrotic syndrome consists of minimal change glomerulonephritis with interstitial nephritis, which is an unusual combination of histologic findings. NSAID-induced nephrotic syndrome without interstitial disease is rare but has been reported in a handful of patients who took fenoprofen, sulindac, or diclofenac. Conversely, interstitial disease without nephrosis has been reported in a few patients, but this may, in fact, represent allergic interstitial nephritis.⁴¹

In spite of nephrotic-range proteinuria, the most impressive histopathologic findings involve the interstitium and tubules. A focal diffuse inflammatory infiltrate can be found around the proximal and distal tubules. We reported that the infiltrate primarily consisted of cytotoxic T lymphocytes but also contained other T cells, B cells, and plasma cells.³⁹ Changes in the glomeruli were minimal and resembled those of minimal change glomerulonephritis with marked epithelial-foot process fusion. Other investigators have reported similar findings.^{1,22,41,42}

The onset of NSAID-induced nephrotic syndrome is usually delayed, having a mean time of onset of 5.4 months after initiation of NSAID therapy⁴⁰ and ranging from 2 weeks to 18 months.¹ NSAID-induced nephrotic syndrome is usually reversible 1 month to 1 year after discontinuation of NSAID therapy. During the recovery period, some patients may require dialysis. Corticosteroids have been used empirically, but it is not clear whether they hasten recovery.^{1,22,39} If proteinuria does not significantly remit within 2 weeks after discontinuation of the NSAID, we recommend a standard, 2-month trial of corticosteroid therapy as would be employed in a nephrotic adult with idiopathic minimal change or membranous glomerulonephritis.

Risk factors are not well understood. Underlying renal impairment does not appear to be a risk factor. Old age has been suggested as a risk factor,^{22,40} but

this may also be a reflection of the usual candidate for chronic NSAID therapy. The syndrome has been more commonly reported with fenoprofen than other NSAIDs. Approximately two-thirds of cases have been associated with fenoprofen. Hence, the structure of the drug itself appears to be of major importance. The syndrome has been attributed, nonetheless, to virtually all NSAIDs, including those from structurally distinct classes.^{1,22,39,40,41}

The mechanism of NSAID-induced nephrotic syndrome has not been fully characterized. The association of this syndrome with structurally distinct NSAIDs suggests a common denominator. T lymphocytes may function as immune mediators instead of the humoral factors that are responsible for classic drug-induced allergic interstitial nephritis. In keeping with this hypothesis, NSAID-induced prostaglandin inhibition may play an indirect role. By inhibiting cyclooxygenase, NSAIDs may promote metabolism of arachidonic acid to non-prostaglandin eicosanoids. Indeed, leukotrienes, the products of the interaction between lipoxygenase and arachidonic acid, are known to recruit T lymphocytes and promote the inflammatory process. Leukotrienes may also contribute to proteinuria by increasing vascular permeability.^{1,40,41}

PAPILLARY NECROSIS

Papillary necrosis with interstitial nephritis is a well-known complication of chronic phenacetin abuse that has been reviewed extensively elsewhere.⁴³ Fortunately, the incidence of the latter complication has diminished considerably because of a better understanding of the pathophysiology and patient education. It has been suggested that chronic aspirin alone may also induce papillary necrosis,⁴⁴ but it is not clear that this can actually occur. What is clinically apparent is that chronic (10 to 20 years) exposure of the kidney to high doses of analgesic combinations such as salicylate and acetaminophen (the metabolite of phenacetin), often with the addition of caffeine, can and will produce chronic, progressive papillary necrosis.

The black pigmentation found within necrotic papillae associated with phenacetin abuse (or phenacetin-containing combinations) is absent in patients who ingest aspirin alone or other NSAIDs. This black pigmentation may represent a breakdown product of phenacetin.⁴⁵

In preclinical studies, nearly all of the NSAIDs produced papillary necrosis in experimental animal models. Clinical toxicity is exceedingly rare but has been reported for ibuprofen,⁴⁶ phenylbutazone,^{46,47}

fenoprofen,⁴⁸ and mefenamic acid,⁴⁹ and according to prescribing information, several other NSAIDs.

The typical candidate for NSAID-induced papillary necrosis is a middle-aged woman with a history of ingesting over-the-counter, combination analgesics for headache. Closer questioning may reveal that the patient takes the analgesic for the mood-altering effects of caffeine. Renal manifestations may include loin pain, macroscopic hematuria, ureteral obstruction, and/or uremia. Urinary tract infection and hypertension are common secondary findings. Reversibility is determined by the extent of deterioration and ability to discontinue NSAID therapy.⁴³ Recent reports from the FDA⁵⁰ of spontaneous gross hematuria associated with NSAIDs such as ibuprofen (three cases) suggest that papillary necrosis also occurs with newer NSAIDs. These data suggest a minor degree of papillary damage, but chronic progressive deterioration of renal function is not a feature of most reports.

The mechanism of NSAID-induced papillary necrosis is not clear. The causative role of NSAIDs is difficult to delineate because of the presence of confounding factors such as underlying disease, urinary tract infection, and/or concomitant medications. Selected NSAIDs may exert a direct toxic effect on renal papillae, particularly combinations of aspirin and acetaminophen, a major metabolite of phenacetin. Both drugs are highly concentrated in the medulla. Aspirin depletes cellular glutathione, which would otherwise neutralize the acetaminophen metabolite, N-acetyl-benzo-quinoneimine. Without glutathione, this highly reactive metabolite could lead to cell death.⁴³

Prostaglandin inhibition may also play a role.¹ Medullary ischemia, a possible precipitating factor in development of papillary necrosis, results from NSAID-induced reduction in blood flow to the renal medulla in experimental models.^{51,52}

OTHER NSAID-INDUCED RENAL SYNDROMES

Phenylbutazone, suprofen, and benoxaprofen produce unique renal syndromes that are of historic interest. These complications are rarely encountered because phenylbutazone use has diminished because of the availability of safer drugs, and suprofen and benoxaprofen have been removed from the market.

Two mechanisms have been identified for phenylbutazone-induced acute oligo-anuric renal failure.¹ Phenylbutazone is known to inhibit uric acid reabsorption, which may cause hyperuricosuria, and ultimately, bilateral ureteral obstruction due to uric acid stones.⁵³ Secondly, an idiosyncratic reaction has

been reported that results in acute tubular injury without uric acid precipitation.⁵⁴ Underlying renal impairment is a risk factor for the latter reaction. Also, patients experiencing this reaction appear to be predisposed to subsequent renal injury from other NSAIDs. These observations suggest that prostaglandin inhibition may play a role in the development of the idiosyncratic reaction.¹

Suprofen-induced acute renal failure is characterized by acute flank and/or abdominal pain, occurring within 12 hours after starting therapy. In a series of 16 patients described by Hart and colleagues,⁵⁵ the mean peak serum creatinine was 3.6 mg/dL (range: 2–8 mg/dL) and was within normal limits at follow-up in most patients. Urinalysis revealed microhematuria (8/12 patients) and proteinuria (7/12 patients) but no crystals. One of our patients with suprofen-induced flank pain syndrome had birefringent crystals in the urine several hours after the injection of the drug and at a time when rehydration had already been commenced. We did not determine if these crystals were uric acid or drug metabolites.

The mechanism of suprofen-induced flank pain and acute renal failure was never conclusively identified before the drug was removed from the market. No obvious risk factors were identified in the previous series since all patients appeared to be in good health and took NSAIDs for acute symptomatic relief. It has been hypothesized that the suprofen flank pain syndrome is related to acute uric acid

crystal precipitation within the nephron leading to acute urinary flow obstruction.^{56,55} Suprofen is known to have uricosuric activity. The finding of hyperuricemia (mean: 10.8 mg/dL) in four of four patients suggests that this may be a risk factor.⁵⁵

Benoxaprofen, an NSAID with a long half-life, was removed from the market in 1982, within weeks after its introduction, because of adverse effects. It is remembered for severe hepatic toxicity that occasionally resulted in death; however, renal failure was also a contributing factor. Risk factors for benoxaprofen-induced toxicity were old age and concomitant diuretic therapy, two factors known to increase the risk of acute functional renal failure.

CONCLUSIONS

NSAIDs are considered safe and suitable for the treatment of a variety of chronic and acute conditions. The risk of renal failure after the initiation of any given NSAID is low; however, the number of at-risk patients is high because of the widespread use of these drugs.

In most cases, NSAID-induced renal syndromes are a direct or indirect result of prostaglandin inhibition, which has important clinical implications. At this time, it is not clear whether it is possible to completely separate the effects of NSAIDs on systemic prostaglandins, which mediate anti-inflammation activity, from renal effects. Thus, under the right cir-

TABLE IV

Summary of Effects of NSAIDs on Renal Function			
Renal Syndrome	Mechanism	Risk Factors	Prevention/Treatment
Sodium retention and edema	↓ Prostaglandin	NSAID therapy (most common adverse effect)	Stop NSAID
Hyperkalemia	↓ Prostaglandin, ↓ potassium to distal tubule and ↓ aldosterone/renin-angiotensin	Renal disease Heart failure Diabetes Multiple myeloma Potassium therapy K ⁺ -sparing diuretic	Stop NSAID Avoid Indomethacin in high-risk patients
Acute deterioration of renal function	↓ Prostaglandin and disruption of hemodynamic balance	Liver disease Renal disease Heart failure Dehydration Old age Fenoprofen	Stop NSAID Avoid use in high-risk patients
Nephrotic syndrome with interstitial nephritis	↑ Lymphocyte recruitment and activation		Stop NSAID Dialysis and (?) steroids as needed
Papillary necrosis	Direct toxicity	Phenacetin abuse Aspirin-acetaminophen combination	Stop NSAID Avoid chronic analgesic use

circumstances, virtually any NSAID can produce renal complications. Fortunately, these complications are usually reversible if the diagnosis is recognized promptly and NSAID therapy is discontinued.

With an understanding of the pathophysiology involved, preventive clinical measures can be put into operation. Risk factors have been identified for most NSAID-induced renal syndromes (Table IV). It is prudent to avoid high-dose, chronic NSAID therapy in at-risk patients (Table III). Unfortunately, this is not always possible. If NSAIDs are necessary in these high-risk groups, the patients should be monitored closely and receive appropriate counselling. Monitoring should begin within a week after initiation of a short-acting NSAID (e.g., ibuprofen) and continue indefinitely for signs of syndromes having delayed onset (e.g., nephrotic syndrome with interstitial nephritis).

In the event of NSAID-induced renal failure, the NSAID should be discontinued promptly. The patient should receive supportive care as needed. After stabilization of renal function, rechallenge with the same dose of the offending drug or even a structurally unrelated NSAID is likely to reproduce the adverse effect. (Patients who have recovered from an episode of protracted dehydration due to diuretics or intercurrent disease are an exception to this rule.) Thus, if anti-inflammatory therapy is mandatory, underlying risk factors should be identified and eliminated, if possible. Unfortunately, this is often not possible, as in the case of old age or chronic heart, kidney, or liver disease. These patients may require alternative therapy using corticosteroids or other supportive drugs such as acetaminophen or colchicine.

REFERENCES

1. Clive DM, Stoff JS: Renal syndromes associated with nonsteroidal anti-inflammatory drugs. *N Engl J Med* 1984;310:563-572.
2. Patrono C, Dunn MJ: The clinical significance of inhibition of renal prostaglandin synthesis. *Kidney Int* 1987;32:1-12.
3. Oates JA, FitzGerald GA, Branch RA, Jackson EK, Knapp HR, Roberts LJ II: Clinical implications of prostaglandin and thromboxane A_2 formation (2 parts). *N Engl J Med* 1988;319:689-698, 761-767.
4. Thame MD, DiBona GF: Renal nerves modulate the secretion of renin mediated by nonneuronal mechanisms. *Circ Res* 1979;44:645-652.
5. Stokes JB: Effect of prostaglandin E_2 on chloride transport across the rabbit thick ascending limb of Henle: Selective inhibition of the medullary portion. *J Clin Invest* 1979;64:495-502.
6. Stokes JB, Kokko JP: Inhibition of sodium transport by prostaglandin E_2 across the isolated, perfused rabbit collecting tubule. *J Clin Invest* 1977;59:1099-1104.
7. Orloff J, Handler JS, Bergstrom S: Effect of prostaglandin (PGE) on the permeability response of the toad bladder to vasopressin, theophylline and adenosine 3', 5'-monophosphate. *Nature* 1965;205:397-398.
8. Anderson RJ, Berl T, McDonald KM, Schrier RW: Evidence for an *in vivo* antagonism between vasopressin and prostaglandin in the mammalian kidney. *J Clin Invest* 1975;56:420-426.
9. Schooley RT, Wagley PF, Lietman PS: Edema associated with ibuprofen therapy. *JAMA* 1977;237:1716-1717.
10. Blum M, Aviram A: Ibuprofen induced hyponatremia. *Rheumatol Rehabil* 1980;19:258-259.
11. Galler M, Folkert VW, Schlendorff D: Reversible acute renal insufficiency and hyperkalemia following indomethacin therapy. *JAMA* 1981;246:154-155.
12. Findling JW, Beckstrom D, Rawsthorne L, Kozin F, Itskovitz H: Indomethacin-induced hyperkalemia in three patients with gouty arthritis. *JAMA* 1980;244:1127-1128.
13. Nicholls MG, Espiner EA: Indomethacin-induced azotaemia and hyperkalaemia: A case study. *N Z Med J* 1981;94:377-379.
14. Paladini G, Tonazzi C: Indomethacin-induced hyperkalemia and renal failure in multiple myeloma. *Acta Haematol (Basel)* 1982;68:256-260.
15. Akbarpour F, Afrasiabi A, Vaziri ND: Severe hyperkalemia caused by indomethacin and potassium supplementation. *South Med J* 1985;78:756-757.
16. Mor R, Pitlik S, Rosenfeld JB: Indomethacin- and Moduretic-induced hyperkalemia. *Isr J Med Sci* 1983;19:533-537.
17. Goldszer RC, Coodley EL, Rosner MJ, Simons WM, Schwartz AB: Hyperkalemia associated with indomethacin. *Arch Intern Med* 1981;141:802-804.
18. MacCarthy EP, Frost GW, Strokes GS: Indomethacin-induced hyperkalaemia. *Med J Aust* 1979;1:550.
19. Tan SY, Shapiro R, Franco R, Stockard H, Mulrow PJ: Indomethacin-induced prostaglandin inhibition with hyperkalemia. *Ann Intern Med* 1979;90:783-785.
20. Berg KJ: Acute effects of acetylsalicylic acid on renal function in normal man. *Eur J Clin Pharmacol* 1977;11:117-123.
21. Donker AJ, Arisz L, Brentjens JR, van der Hem GK, Holleman HJ: The effect of indomethacin on kidney function and plasma renin activity in man. *Nephron* 1976;17:288-296.
22. Blackshear JL, Napier JS, Davidman M, Stillman MT: Renal complications of nonsteroidal anti-inflammatory drugs: Identification and monitoring of those at risk. *Semin Arthritis Rheum* 1985;14:163-175.
23. Zipser RD, Hoefs JC, Speckart PF, Zia PK, Horton R: Prostaglandins: Modulators of renal function and pressor resistance in chronic liver disease. *J Clin Endocrinol Metab* 1979;48:895-900.
24. Zipser RD, Radvan GH, Kronborg J, Duke R, Little TE: Urinary thromboxane B_2 and prostaglandin E_2 in the hepatorenal syndrome: Evidence for increased vasoconstrictor and decreased vasodilator factors. *Gastroenterology* 1983;84:697-703.
25. Walshe JJ, Venuto RG: Acute oliguric renal failure induced by indomethacin: Possible mechanisms. *Ann Intern Med* 1979;91:47-49.
26. Arisz L, Donker AJM, Brentjens JRH, van der Hem GK: The effect of indomethacin on proteinuria and kidney function in the nephrotic syndrome. *Acta Med Scand* 1976;199:121-125.
27. Kleinknecht C, Broyer M, Gubler M-C, Palcoux J-B: Irreversible renal failure after indomethacin in steroid-resistant nephrosis. *N Engl J Med* 1980;302:691.
28. Kimberly RP, Gill JR Jr, Bowden RE, Keiser HR, Plotz PH: Elevated urinary prostaglandins and the effects of aspirin on renal function in lupus erythematosus. *Ann Intern Med* 1978;89:336-341.

29. Fong HJ, Cohen AH: Ibuprofen-induced acute renal failure with acute tubular necrosis. *Am J Nephrol* 1982;2:28-31.

30. Whelton A, Stout RL, Spilman PS, Klassen DK: Renal effects of ibuprofen, piroxicam, and sulindac in patients with asymptomatic renal failure: A prospective, randomized, crossover comparison. *Ann Intern Med* 1990;112:568-576.

31. Favre L, Glasson P, Vallotton MB: Reversible acute renal failure from combined triamterene and indomethacin: A study in healthy subjects. *Ann Intern Med* 1982;96:317-320.

32. McCarthy JT, Torres VE, Romero JC, Wochos DN, Velosa JA: Acute intrinsic renal failure induced by indomethacin: Role of prostaglandin synthetase inhibition. *Mayo Clin Proc* 1982;57:289-296.

33. Bunning RD, Barth WF: Sulindac: A potentially renalsparing nonsteroidal anti-inflammatory drug. *JAMA* 1982;248:2864-2867.

34. Brater DC, Anderson S, Baird B, Campbell WB: Effects of ibuprofen, naproxen, and sulindac on prostaglandins in men. *Kidney Int* 1985;27:68-73.

35. Sedor JR, Williams SL, Chremos AN, Johnson CL, Dunn MJ: Effects of sulindac and indomethacin on renal prostaglandin synthesis. *Clin Pharmacol Ther* 1984;36:85-91.

36. Ciabattoni G, Cinotti GA, Pierucci A, et al: Effects of sulindac and ibuprofen in patients with chronic glomerular disease: Evidence for the dependence of renal function on prostacyclin. *N Engl J Med* 1984;310:279-283.

37. Mistry CD, Lote CJ, Gokal R, Currie WJ, Vandenberg M, Mallick NP: Effects of sulindac on renal function and prostaglandin synthesis in patients with moderate chronic renal insufficiency. *Clin Sci* 1986;70:501-505.

38. Quintero E, Ginés P, Arroyo V, et al: Sulindac reduces the urinary excretion of prostaglandins and impairs renal function in patients with cirrhosis and ascites. *Nephron* 1986;42:298-303.

39. Bender WL, Whelton A, Beschorner WE, Darwish MO, Hall-Craggs M, Solez K: Interstitial nephritis, proteinuria, and renal failure caused by nonsteroidal anti-inflammatory drugs: Immunologic characterization of the inflammatory infiltrate. *Am J Med* 1984;76:1008-1012.

40. Abraham PA, Keane WF: Glomerular and interstitial disease induced by nonsteroidal anti-inflammatory drugs. *Am J Nephrol* 1984;4:1-6.

41. Levin ML: Patterns of tubulo-interstitial damage associated with nonsteroidal anti-inflammatory drugs. *Semin Nephrol* 1988;8:55-61.

42. Stachura I, Jayakumar S, Bourke E: T + B lymphocyte subsets in fenoprofen nephropathy. *Am J Med* 1983;75:9-16.

43. Kincaid-Smith P: Effects of non-narcotic analgesics on the kidney. *Drugs* 1986;32(Suppl. 4):109-128.

44. Krishnaswamy S, Nanra RS: "Phenacetin" nephropathy without phenacetin (abstract). *Aust NZ J Med* 1976;6:88.

45. Shah GM, Muhalwas KK, Winer RL: Renal papillary necrosis due to ibuprofen. *Arthritis Rheum* 1981;24:1208-1210.

46. Lourie SH, Denman SJ, Schroeder ET: Association of renal papillary necrosis and ankylosing spondylitis. *Arthritis Rheum* 1977;20:917-921.

47. Morales A, Steyn J: Papillary necrosis following phenylbutazone ingestion. *Arch Surg* 1971;103:420-421.

48. Husserl FE, Lange RK, Kantrow CM Jr: Renal papillary necrosis and pyelonephritis accompanying fenoprofen therapy. *JAMA* 1979;242:1896-1898.

49. Robertson CE, Ford MJ, Van Someren V, Dlugolecka M, Prescott LF: Mefenamic acid nephropathy. *Lancet* 1980;2:232-233.

50. Harter JG: Acute flank pain and hematuria: Lessons from adverse drug reaction reporting. *J Clin Pharmacol* 1988;28:560-565.

51. Kirschenbaum MA, White N, Stein JH, Ferris TF: Redistribution of renal cortical blood flow during inhibition of prostaglandin synthesis. *Am J Med* 1974;227:801-805.

52. Stein JH, Fadem SZ: The renal circulation. *JAMA* 1978;239:1308-1312.

53. Weisman JL, Bloom B: Anuria following phenylbutazone therapy. *N Engl J Med* 1955;252:1086-1087.

54. Lipsett MB, Goldman R: Phenylbutazone toxicity: Report of a case of acute renal failure. *Ann Intern Med* 1954;41:1075-1079.

55. Hart D, Ward M, Lifschitz MD: Suprofen-related nephrotoxicity. A distinct clinical syndrome. *Ann Intern Med* 1987;106:235-238.

EXHIBIT L

The Renal Effects of Nonsteroidal Anti-inflammatory Drugs: Summary and Recommendations

William M. Bennett, MD, William L. Henrich, MD, and Jeffrey S. Stoff, MD

- The renal effects of nonsteroidal anti-inflammatory drugs are reviewed with special emphasis on the clinical, pathophysiologic, and risk factors for acute renal failure. Renal papillary necrosis and chronic renal insufficiency can occur with the prolonged use of these drugs, although the prevalence of this manifestation of nonsteroidal anti-inflammatory drug nephrotoxicity is unknown. Current recommendations based on a critical literature survey are provided, along with a list of suggested areas in which more research is needed.

© 1996 by the National Kidney Foundation, Inc.

INDEX WORDS: Nonsteroidal anti-inflammatory drugs; acute renal failure; chronic renal failure; analgesic nephropathy; prostaglandins.

NONSTEROIDAL anti-inflammatory drugs (NSAIDs) are popular and used widely because of their acknowledged efficacy and excellent safety profile in a wide range of clinical conditions. Despite their many useful therapeutic applications, there is now substantial evidence arising from experimental studies and clinical studies in humans for multiple effects of NSAIDs on kidney function. This is not surprising since the principal action of NSAIDs is to block the synthesis of cyclo-oxygenase products of arachidonic acid, which have a critical modulatory role on renal hemodynamics, renal epithelial cell fluid and ion transport, and the synthesis and action of renal hormones. Nonsteroidal anti-inflammatory drugs are now available both in over-the-counter and prescription strengths. The majority of healthy, normal subjects who ingest therapeutic dosages of NSAIDs for limited duration tolerate these drugs without adverse effects. However, a subset of individual are susceptible to subclinical as well as serious renal toxicity from these agents. In addition to the effects listed in Table 1, NSAIDs interfere with the efficacy of antihypertensive medicines, leading to an increase in blood pressure.

Since the toxicity of NSAIDs in the kidney is linked to the disruption of renal prostaglandin

synthesis, a brief review of the renal effects of prostaglandins and the consequences of synthesis interruption is in order.

PROSTAGLANDIN SYNTHESIS AND COMPARTMENTALIZATION

Prostaglandins are derivatives of arachidonic acid, a 20-carbon tetraenoic acid, which is acylated to membrane phospholipids. Deacylation of arachidonic acid from the cell membrane is controlled by phospholipases, predominantly phospholipase A₂. Vasopressin,¹ bradykinin,² angiotensin,³ and norepinephrine⁴ all stimulate arachidonic acid release from membranes, whereas glucocorticoids inhibit release.⁵ After arachidonic acid is released from the cell membrane, several synthetic pathways are then available. Molecular oxygen may be added to the arachidonic acid by the action of an intracellular endoplasmic reticulum-bound peroxidase enzyme (cyclo-oxygenase), which leads to the synthesis of endoperoxide PGG₂. A second endoperoxide (PGH₂) is then formed with the liberation of a superoxide radical. Once formed, PGH₂ has a short half-life and is rapidly acted on by a series of enzymes that produce the biologically active molecules. Nonsteroidal anti-inflammatory drugs exert their prostaglandin inhibitory effects by primarily inhibiting the activity of cyclo-oxygenase by 70% to 95%. Prostaglandin biosynthesis is also decreased by NSAIDs, reducing the generation of superoxide and hydroxyl-free radicals.^{6,7}

The endoperoxide PGH₂ is transformed by a series of enzymes to the dienoic series of prostaglandins. These prostaglandin metabolites possess biologic activity in the kidney; for example, prostacyclin synthetase acts to form prostacyclin (PGI₂), whereas thromboxane synthetase forms thromboxane (TXA₂) and the isomerases act to

From the Department of Medicine, Oregon Health Sciences University, Portland, OR; the Department of Medicine, Medical College of Ohio, Toledo, OH; and the Department of Medicine, University of Massachusetts Medical Center, Worcester, MA.

Address reprint requests to William M. Bennett, MD, Oregon Health Sciences University, Division of Nephrology and Hypertension, 3314 SW US Veterans Hospital Rd, PP262, Portland, OR 97201-2940.

© 1996 by the National Kidney Foundation, Inc.
0272-6386/96/2801-0111\$3.00/0

Table 1. Kidney Manifestations of NSAIDs

Kidney Toxicity	Mechanism	Risk Factors
Acute renal failure	Loss of counterregulatory prostaglandins	Plasma volume contraction, congestive heart failure, cirrhosis, and ascites
Sodium retention	Loss of natriuretic prostaglandins	Unknown
Potassium retention	Hyporeninemic hypoaldosteronism	Concomitant defects in potassium homeostasis
Water retention	Enhanced antidiuretic hormone action, increased medullary tonicity	Unknown
Acute interstitial nephritis	Reactive arachidonic acid metabolite	Unknown

form PGE₂ and PGF₂. Prostaglandins are known to exert physiologic effects at the locations at which they are synthesized. In this regard, they are really autocoids rather than true hormones. Prostaglandins that are excreted into renal lymph or into the renal vein are rapidly metabolized into active products in the lung. The prostaglandin synthetic pathway is shown in Fig 1. Prostaglandins synthesized in the renal cortex regulate renal cortical processes (renal vascular resistance and renal secretion), whereas prostaglandins formed in the medulla modulate medullary physiologic events (salt and water handling). The most abundant prostaglandin found in the tubules is PGE₂. The cortical and particularly medullary portion of the collecting duct are the dominant sites of PGE₂ synthesis. Medullary interstitial cells are also a rich source of PGE₂ production. Prostaglandin E₂ undergoes spontaneous hydrolysis to 6-keto-PGF_{1α}. Prostaglandins are rapidly metab-

olized into inactive products by a 15-prostaglandin dehydrogenase.

EFFECTS OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS ON RENAL FUNCTION: CLINICAL CONSEQUENCES

Under baseline and euvolemic circumstances there is typically a very low rate of prostaglandin synthesis. Because this is true in a healthy state, it is difficult to demonstrate that prostaglandins contribute to the normal maintenance of renal function even when using powerful cyclo-oxygenase inhibitors, such as NSAIDs. When prostaglandin synthesis is upregulated as hemodynamic destabilization occurs, the synthesis and release of prostaglandins is greatly enhanced. Under these circumstances the inhibition of prostaglandin synthesis has been clearly demonstrated to have profound adverse hemodynamic effects on the kidney. Most of these effects have been seen

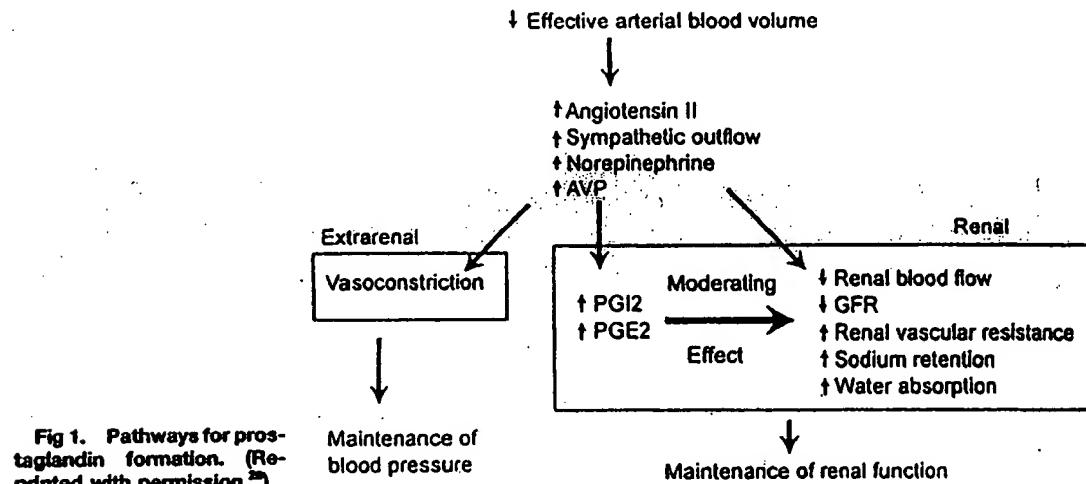


Fig 1. Pathways for prostaglandin formation. (Reprinted with permission.²⁹)

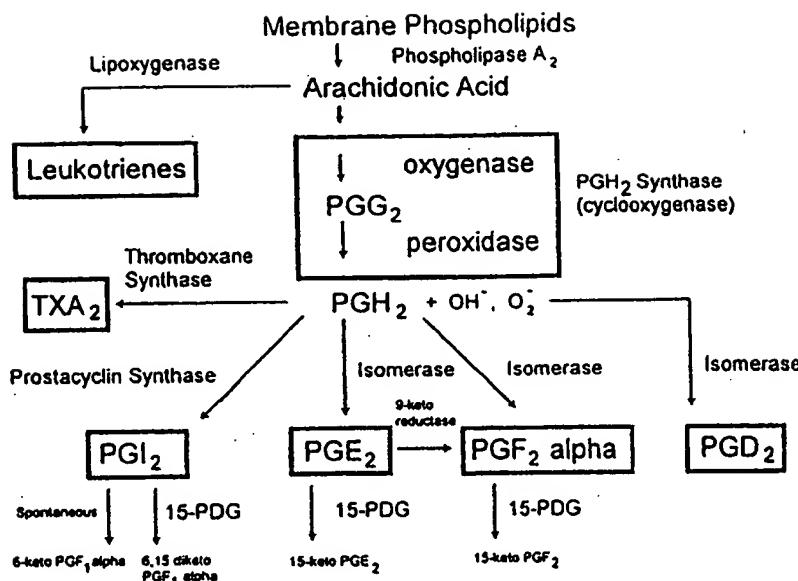


Fig 2. Schematic depiction of the relationship between vasodilator and vasoconstrictor input into the kidney. PGI₂ and PGE₂ exert a moderating effect on renal vasoconstrictive stimuli.

in circumstances in which blood volume or effective arterial blood volume is compromised and vasoconstrictor peptide secretion would be expected to be high. Angiotensin II, norepinephrine, vasopressin, and sympathetic nerve activity all increase under these perturbed circumstances and cause an increase in renal vascular resistance. In addition, each of these stimuli is a potent agonist for prostaglandin synthesis.^{2,3,6} Hence, what ensues is a dynamic interplay between counterbalancing vasoconstrictor and vasodilator forces. It is under these circumstances that the inhibitor of prostaglandin synthesis will result in excessive vasoconstriction, with a consequent decrease in renal blood flow and finally a decrement in glomerular filtration rate. These relationships are graphically depicted in Fig 2.

ACUTE RENAL FAILURE

Acute renal failure (ARF) due to a decrease in renal blood flow secondary to increased renal vascular resistance has been well described. The afferent renal arteriole appears to be under tonic regulation by vasodilator prostaglandins, and loss of these dilators leads to vasoconstriction and a decrease in glomerular capillary pressure, resulting in a prompt decline in glomerular filtration rate. This form of renal failure is often sudden, presenting with oliguria and a decrease in

fractional sodium excretion. Withdrawal of NSAIDs usually leads to prompt reversal of the ARF. Virtually all NSAIDs have been implicated, although some subclasses of NSAIDs may be less toxic because of renal conversion of active drug to inactive metabolite.

A common risk factor of ARF is the physiologic state of plasma volume depletion induced either by hemorrhage, salt loss, or hypoalbuminemia. In these conditions, circulating vasoconstrictors are released, maintaining vascular resistance and blood pressure at the potential expense of regional organ blood flow. To maintain blood flow, particularly in the kidney, counterregulatory renal prostaglandins are released that counteract vasoconstrictors and normalize renal blood flow. Nonsteroidal anti-inflammatory drugs taken under these circumstances blunt this counterregulatory response and intensify the renal vasoconstriction leading to ARF. If the vasoconstriction is sufficiently intense and of extended duration, acute tubular necrosis may ensue. Similar physiology to intravascular volume depletion is seen in severe congestive heart failure (New York Heart Association grade III, IV) and hepatic failure with ascites. In these two pathophysiologic states, which are also associated with activation of circulating neurohumoral vasoconstrictors, NSAID use may lead to ARF by augmenting arteriolar constriction.

INTERRUPTION OF RENAL TUBULAR ION AND WATER TRANSPORT: CLINICAL CONSEQUENCES

Eicosanoids or oxygenated metabolites of arachidonic acid exert modulatory influences on many ion transport sites along the nephron. Consequently, their synthesis interrupted by NSAID use leads to a wide variety of disorders of ion transport. Most prominent among these in clinical use is the retention of sodium. Virtually all individuals will develop positive sodium retention following the use of NSAIDs and escape from this antinatriuretic effect in several days. A small subset of individuals fail to escape and develop a severe edema state. Natriuresis rapidly ensues once the drug is discontinued.

An issue related to sodium retention is the effect of NSAIDs to antagonize the effect of concomitant diuretic use. This antagonism has been described for the use of both thiazide and loop diuretics. Potassium-sparing diuretics, particularly triamterene, have been implicated as a potential risk factor for NSAID-induced ARF. Reports of this combination of drugs have been in the form of case reports and require further study to document the precise risk.

Hyperkalemia is the second major electrolyte disorder that accompanies NSAID use. Since plasma potassium is tightly regulated by several different effector systems, NSAID-induced hyperkalemia seldom occurs in the absence of other defects in potassium homeostasis. The mechanism of NSAID action is the suppression of prostaglandin-mediated renin release leading to a state of hyporeninemic hypoaldosteronism. Patients at risk are those on drugs that block internal potassium homeostasis (beta blockers, alpha agonists) or drugs that reduce potassium excretion (potassium-sparing diuretics, aldosterone antagonists). Insulin-dependent diabetic patients, especially with renal dysfunction, as well as patients with moderate to severe renal failure (glomerular filtration rate < 30 mL/min) are at particularly high risk.

Hyponatremia secondary to a defect in free water clearance is well documented in the use of NSAIDs. Abundant evidence indicates that prostaglandins antagonize the hydro-osmotic effect of antidiuretic hormone. Thus, NSAID use enhances antidiuretic hormone action and promotes water retention. This effect is further accentuated

by the effect of NSAIDs to augment medullary tonicity by enhancing the active transport of chloride at the thick ascending limb of the loop of Henle. Restriction of water intake may be necessary in those patients who develop hyponatremia during NSAID use.

ACUTE INTERSTITIAL NEPHRITIS AND MINIMAL-CHANGE GLOMERULOPATHY

Nonsteroidal anti-inflammatory drugs of all classes have been reported to induce a syndrome of acute interstitial nephritis with or without minimal-change glomerulopathy. This rare syndrome has been reported after 2 to 18 months of NSAID therapy and may be sufficiently severe as to require dialysis support. Most cases are reversible and are characterized pathologically by a mononuclear cell infiltrate of lymphocytes and plasma cells. When there is glomerular involvement, the predominant lesion is epithelial cell podocyte fusion detected by electron microscopy. The most culpable NSAID appears to be fenoprofen, although virtually all NSAIDs have been reported to induce this pathology. Acute interstitial nephritis is probably the most common presentation, followed by combined interstitial and glomerular disease; the least common is minimal-change glomerulopathy. The usual stigmata of an allergic syndrome are absent, such as skin rash, peripheral eosinophilia, and increased immunoglobulin E level, suggesting that the mechanism of action may be related to a reactive non-cyclo-oxygenase product of arachidonic acid metabolism. The syndrome is usually reversible by the withdrawal of the offending NSAID. There are no controlled studies supporting the use of corticosteroids to alter the rate or extent of renal recovery.

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AND CHRONIC RENAL DISEASE

Despite the well-characterized acute biologic effects of NSAIDs on the kidney, there are no scientifically acceptable data documenting the safety of this class of drugs on renal structure and function when taken chronically. Epidemiologic data show an 8.8 increased relative risk of end-stage renal disease in subjects ingesting 5,000 or more doses of NSAIDs compared with control subjects matched for age. However, these data are flawed by the study design and do not neces-

sarily support a cause and effect relationship.⁹ In a better-designed, multicenter, case control study, the risk of chronic renal disease defined as a serum creatinine of ≥ 1.5 mg/dL was 2.1 (95% confidence interval, 1.1 to 4.1) in daily users of NSAIDs.¹⁰

The hallmark lesion of analgesic-associated nephropathy is renal papillary necrosis, which can lead to progressive renal failure but also may be present with a well-preserved glomerular filtration rate, making ascertainment of cases by renal function studies alone problematic. In a prospective radiographic study of 259 patients with an intake of 1,000 to 26,000 NSAID doses, papillary necrosis was found in 38 users who took predominately physician-prescribed NSAIDs. Only 65% of these patients had renal functional impairment. Thus, it is clear that long-term use of NSAIDs can cause renal papillary necrosis and renal insufficiency.¹¹ The frequency of renal papillary necrosis as a primary or contributing cause of end-stage renal disease is unknown because of the infrequent radiographic diagnosis by physicians resulting in misclassification and the insensitivity of renal diagnosis by currently available renal function tests, such as serum creatinine. Furthermore, other known effects of NSAIDs on the kidney, including increased blood pressure and renal hemodynamic changes, could contribute to facilitating progressive renal disease of other etiologies. The experimental production of renal papillary necrosis by NSAIDs is enhanced by caffeine.¹² It is not known whether this is clinically relevant because caffeine intake has not been considered in epidemiologic or other clinical studies.

While there is an extensive package insert documenting the renal consequences of prescription NSAIDs, there are no renal warnings at all on over-the-counter NSAIDs, which are heavily advertised to the public. Thus, patients in high-risk groups or patients with pre-existing kidney disease could be unaware that they have been exposed to these drugs. Case reports and case series document the ability of a variety of chemically unrelated NSAIDs to produce renal papillary necrosis and renal insufficiency.¹³⁻¹⁶

There are other causes of chronic renal failure in patients using prescription or over-the-counter NSAIDs. Although acute renal dysfunction due to NSAIDs is most often reversible, approximately 20% of reported cases have permanent

renal failure whether the NSAIDs produced ARF via acute tubular necrosis, acute interstitial nephritis with proteinuria, or simply renal blood flow decreases in high-risk populations.¹⁷

Irreversible renal failure may also occur in children.¹⁸ Prenatal exposure to indomethacin may lead to severe irreversible renal failure, which is favored by prior stimulation of the renin-angiotensin system. Since these infants are not generally candidates for renal replacement, the consequences of NSAIDs in this setting are not reflected in the end-stage renal disease statistics of the US Renal Data System. In recent series, neonatal renal failure deaths were reported with 150 to 400 mg of indomethacin per day for 2 to 11 weeks during pregnancy.¹⁹⁻²² Low birth weights and hyperkalemia also have been described in surviving infants.²³

When NSAIDs are used to reduce proteinuria in nephrosis, permanent renal damage has been reported. Another potential adverse effect of NSAIDs in patients with chronic renal failure includes fatal hyperkalemia from drug-drug interactions with angiotensin-converting enzyme inhibitors, potassium-sparing diuretics, or beta blockers.²⁴

Although the population exposure to prescription and nonprescription NSAIDs is large, even the estimated 1% patients with clinically detectable renal dysfunction has important medical and economic implications.²⁵ The longest period of observation with regard to chronic NSAID usage is 6 to 12 months. In the United States, there are no cross-sectional or prospective studies applied to NSAIDs using the objective criteria for analgesic nephropathy diagnosis proposed by Elseviers and DeBroe,²⁶ although these criteria have been validated in Europe. In a large general internal medicine practice in which records of analgesic users were surveyed, patients older than 65 years and those with coronary artery disease were at risk of renal impairment with NSAIDs compared with users of acetaminophen. No radiographic data are available.²⁷

SUMMARY

Nonsteroidal anti-inflammatory use in the general population is safe and efficacious when used in therapeutic dosages for a limited period of time. In contrast, patients with pre-existing risk factors are susceptible to potentially life-threat-

ening toxicities, including ARF and serious fluid and electrolyte disorders. Numerous studies have delineated the mechanism(s) by which NSAIDs induce these adverse effects and identify the patients at highest risk (Table 1). The safe use of these agents requires the identification of these risk factors, interventions to ameliorate these risks when possible, and the careful monitoring of renal function and electrolyte concentrations to avoid serious complications.

Renal papillary necrosis and chronic renal insufficiency can occur secondary to prolonged use of prescription and over-the-counter NSAIDs. Neonatal renal failure and renal death may occur from use during pregnancy. While acute renal failure due to NSAIDs occurs in well-defined high-risk patients or under rare idiosyncratic circumstances, renal recovery is incomplete in approximately 20% of reported cases. There are epidemiologic data to support NSAID use as a risk factor for chronic renal dysfunction, even end-stage renal disease, in a cumulative dose-dependent fashion. Despite over-the-counter status, there are no long-term studies of renal structure or function that document the safety of these drugs.

CONCLUSIONS

1. Use of NSAIDs in the general population is safe and effective when used in therapeutic dosages for a limited period of time.
2. Patients with pre-existing risk factors are susceptible to potentially life-threatening toxicities, including ARF and serious fluid and electrolyte disorders.
3. Renal papillary necrosis and chronic renal failure can occur secondary to prolonged use of prescription and over-the-counter NSAIDs.
4. Neonatal renal failure and renal death may occur from NSAID use during pregnancy.
5. Nonsteroidal anti-inflammatory drug-induced ARF is usually, but not inevitably, reversible.
6. There are no acceptable epidemiologic or clinical data regarding the risk of NSAIDs for chronic renal failure, renal papillary necrosis, or end-stage renal disease.
7. There are no data of NSAIDs' effect on progression of other renal diseases (experimental or clinical).

RECOMMENDATIONS

1. There should be an explicit label to warn patients taking over-the-counter NSAIDs of potential renal toxicities (similar to that suggested in *Am J Kidney Dis* 6:4-5, 1985).
2. Design and implement properly controlled studies on the renal and cardiovascular safety of chronic NSAIDs by themselves or in the presence of other known etiologies of renal disease.
3. Combinations of NSAIDs with other analgesics and/or caffeine should be prospectively evaluated for renal safety prior to release.

REFERENCES

1. Dunn MJ, Hood VL: Prostaglandins and the kidney. *Am J Physiol* 233:F169-184, 1977
2. McGiff JC, Crowshaw K, Terragno NA, Malik KU, Lonigro AJ: Differential effect of noradrenaline and renal nerve stimulation on vascular resistance in the dog kidney and the release of a prostaglandin E-like substance. *Clin Sci* 42:233, 1972
3. McGiff JC, Crowshaw K, Terragno NA, Lonigro AJ: Release of a prostaglandin-like substance into renal venous blood in response to angiotensin II. *Circ Res* 27:121-130, 1970 (suppl 1)
4. Levine L, Moskowitz MA: Alpha and beta adrenergic stimulation of arachidonic acid metabolism cells in culture. *Proc Natl Acad Sci U S A* 76:6632-6636, 1979
5. Zusman RM, Keiser HR: Prostaglandin biosynthesis by rabbit renomedullary interstitial cells in tissue culture; stimulation by angiotensin II, bradykinin, and vasopressin. *J Clin Invest* 60:215-223, 1970
6. McCord JM, Fridovich I: The biology and pathology of oxygen radicals. *Ann Intern Med* 89:122-127, 1978
7. Simon LS, Mills JA: Nonsteroidal anti-inflammatory drugs. *N Engl J Med* 302:1179-1185, 1980
8. McGiff JC, Crowshaw K, Terragno NA, Lonigro AJ: Renal prostaglandins: Possible regulators of the renal actions of pressor hormones. *Nature* 227:1255-1257, 1970
9. Perneger TV, Whelton PK, Klag MJ: Risk of kidney failure associated with the use of acetaminophen, aspirin, and nonsteroidal antiinflammatory drugs. *N Engl J Med* 331:1675-1679, 1994
10. Sandler DP, Burr FR, Weinberg CR: Nonsteroidal anti-inflammatory drugs and the risk for chronic renal disease. *Ann Intern Med* 115:165-172, 1991
11. Segalothy M, Samad SA, Zulfiqar A, Beamer WM: Chronic renal disease and papillary necrosis associated with the long-term use of nonsteroidal anti-inflammatory drugs as the sole or predominant analgesic. *Am J Kidney Dis* 24:17-24, 1994
12. Champion de Crespigny P, Hewitson T, Birchall I, Kincaid-Smith P: Caffeine potentiates the nephrotoxicity of mefenamic acid on the rat renal papilla. *Am J Nephrol* 10:311-315, 1990
13. Giovannini JL, Orr H, de Torrente A: Tenoxicam and renal function. Short-term and long-term prospective studies. *J Suisse Med* 120:793-797, 1990

14. Calvo-Alen J, De Cos MA, Rodriguez-Valverde V, Escallada R, Florez J, Arias M: Subclinical renal toxicity in rheumatic patients receiving long-term treatment with nonsteroidal antiinflammatory drugs. *J Rheumatol* 21:1742-1747, 1994
15. Adam O, Vetter-Kerhoff C, Schlondorff D: Renal side-effects of non-steroidal antirheumatic drugs. *Med Klin* 89:305-311, 1994
16. Nanra RS: Analgesic nephropathy in the 1990s—An Australian perspective. *Kidney Int* 42:S86-92, 1993
17. Shibusaki T, Ishimoto F, Sakai O, Joh K, Aizawa S: Clinical characterization of drug-induced allergic nephritis. *Am J Nephrol* 11:174-180, 1991
18. Lantz B, Cochat P, Bouchet JL, Fischbach M: Short-term niflumic-acid-induced acute renal failure in children. *Nephrol Dial Transplant* 9:1234-1239, 1994
19. van der Heijden BJ, Carlus C, Narcy F, Bavoux F, Delezoide AL, Gubler MC: Persistent anuria, neonatal death, and renal microcystic lesions after prenatal exposure to indomethacin. *Am J Obstet Gynecol* 171:617-623, 1994
20. Gloor JM, Muchani DG, Norling LL: Prenatal maternal indomethacin use resulting in prolonged neonatal renal insufficiency. *J Perinatol* 13:425-427, 1993
21. Kaplan BS, Restaino I, Raval DS, Gottlieb RP, Bernstein J: Renal failure in the neonate associated with in utero exposure to non-steroidal anti-inflammatory agents. *Pediatr Nephrol* 8:700-704, 1994
22. Jacqz-Aigrain E, Guillonneau M, Boissinot C, Bavoux F, Hartmann JF, Blot P: Maternal and neonatal effects of indomethacin administrated during pregnancy. Apropos of 18 cases. *Arch Fr Pediatr* 50:307-312, 1993
23. Nishikubo T, Takahashi Y, Nakagawa Y, Kawaguchi C, Nakajima M, Ichijo M, Yoshioka A: Renal impairment in very low birthweight infants following antenatal indomethacin administration. *Acta Paediatr Jpn* 36:202-206, 1994
24. Murray MD, Brater DC: Renal toxicity of the nonsteroidal anti-inflammatory drugs. *Annu Rev Pharmacol Toxicol* 33:435-465, 1993
25. Whelton A, Hamilton CW: Nonsteroidal anti-inflammatory drugs: Effects on kidney function. *J Clin Pharmacol* 31:588-598, 1991
26. Elseviers MM, DeSchepper A, Corthouts R, Bosmans JL, Cosyn L, Lins RL, Lornoy W, Matthys E, Roose R, Van Caesbroeck D, Waller I, Horackova M, Schwarz A, Svrek P, Bonucci D, Franek E, Morlans M, De Broe ME: High diagnostic performance of CT scan for analgesic nephropathy in patients with incipient to severe renal failure. *Kidney Int* 48:1316-1323, 1995
27. Murray MD, Brater DC, Tierney WM, Hui SL, McDonald CJ: Ibuprofen-associated renal impairment in a large general internal medicine practice. *Am J Med Sci* 299:222-229, 1990
28. Palmer B, Henrich W: Systemic complications of nonsteroidal antiinflammatory drug use, in Schrier RW (ed): *Advances in Internal Medicine*. Chicago, IL, Mosby, 1996, pp 605-639

EXHIBIT M

NCBI
20

PubMed

About Entrez

卷之三

Text Version 1: Prog Drug Res. 1997;49:155-71.

Entrez PubMed
Overview Help | FAQ
Tutorial New/Noteworthy
E-Utilities

The diagram illustrates the relationship between E-Utilities and various PubMed services. E-Utilities is positioned on the left, connected by arrows to several services on the right: PubMed Services, Journals Database, MeSH Database, Single Citation Match, Batch Citation Match, Clinical Queries, and LinkOut. The services on the right are also interconnected by arrows.

```
graph LR; EUtilities[E-Utilities] --> PubMedServices[PubMed Services]; EUtilities --> JournalsDatabase[Journals Database]; EUtilities --> MeSHDatabase[MeSH Database]; EUtilities --> SingleCitationMatch[Single Citation Match]; EUtilities --> BatchCitationMatch[Batch Citation Match]; EUtilities --> ClinicalQueries[Clinical Queries]; EUtilities --> LinkOut[LinkOut]; PubMedServices --> JournalsDatabase; PubMedServices --> MeSHDatabase; PubMedServices --> SingleCitationMatch; PubMedServices --> BatchCitationMatch; PubMedServices --> ClinicalQueries; PubMedServices --> LinkOut; JournalsDatabase --> MeSHDatabase; JournalsDatabase --> SingleCitationMatch; JournalsDatabase --> BatchCitationMatch; JournalsDatabase --> ClinicalQueries; JournalsDatabase --> LinkOut; MeSHDatabase --> SingleCitationMatch; MeSHDatabase --> BatchCitationMatch; MeSHDatabase --> ClinicalQueries; MeSHDatabase --> LinkOut; SingleCitationMatch --> BatchCitationMatch; SingleCitationMatch --> ClinicalQueries; SingleCitationMatch --> LinkOut; BatchCitationMatch --> ClinicalQueries; BatchCitationMatch --> LinkOut; ClinicalQueries --> LinkOut;
```

- Related Resources
- Order Documents
- NLM Catalog
- NLM Gateway
- TOXNET
- Consumer Health
- Clinical Alerts
- ClinicalTrials.gov
- PubMed Central

Duplication Timers

- Review Decision Types.

PMID: 9388387 [PubMed - indexed for MEDLINE]

Display Abstract Sort Show: 20 Send To Text

<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&listuids=9388387>

11/12/2004

[Write to the Help Desk](#)

[NCBI](#) | [NLM](#) | [NIH](#)

[Department of Health & Human Services](#)

[Privacy Statement](#) | [Freedom of Information Act](#) | [Disclaimer](#)

Nov 8 2004 18:23:56